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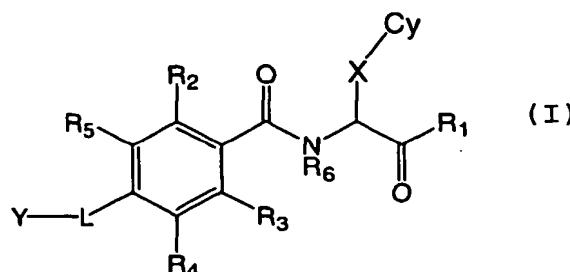
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(54) Title: LFA-1 ANTAGONIST COMPOUNDS



(57) Abstract: The invention relates to novel compounds having formula (I), wherein Cy, X, Y, L and R1-6 are as defined herein. The compounds bind CD11/CD18 adhesion receptors such as Lymphocyte Function-associated Antigen-1 (LFA-1) and are therefore useful for treating disorders mediated by LFA-1 such as inflammation

WO 02/059114 A1

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LFA-1 ANTAGONIST COMPOUNDS

FIELD OF THE INVENTION

15

The invention relates to novel compounds which bind CD11/CD18 adhesion receptors, in particular Lymphocyte Function-associated Antigen-1 (LFA-1) as well as pharmaceutical compositions containing these compounds
20 which are useful for treating disorders mediated thereby.

BACKGROUND OF THE INVENTION

25

Inflammation

Human peripheral blood is composed principally of red blood cells, platelets and white blood cells or leukocytes. The family of leukocytes are further classified as neutrophils, lymphocytes (mostly B- and T-cell subtypes), monocytes, eosinophils and basophils.
30 Neutrophils, eosinophils and basophils are sometimes referred to as "granulocytes" or "polymorphonuclear (PMN) granulocytes" because of the appearance of granules in their cytoplasm and their multiple nuclei. Granulocytes and monocytes are often classified as "phagocytes" because of their ability to phagocytose or ingest micro-organisms and foreign matter referred to generally as "antigens". Monocytes are so called because of their

5 large single nucleus and these cells may in turn become macrophages. Phagocytes are important in defending the host against a variety of infections and together with lymphocytes are also involved in inflammatory disorders.
10 The neutrophil is the most common leukocyte found in human peripheral blood followed closely by the lymphocyte. In a microliter of normal human peripheral blood, there are about 6,000 leukocytes, of which about 4,000 are neutrophils, 1500 are lymphocytes, 250 are monocytes, 150 are eosinophils and 25 are basophils.

15 During an inflammatory response peripheral blood leukocytes are recruited to the site of inflammation or injury by a series of specific cellular interactions (see Fig. 1). The initiation and maintenance of immune functions are regulated by intercellular adhesive interactions as well as signal transduction resulting from interactions between leukocytes and other cells.
20 Leukocyte adhesion to vascular endothelium and migration from the circulation to sites of inflammation is a critical step in the inflammatory response (Fig. 1). T-cell lymphocyte immune recognition requires the interaction of the T-cell receptor with antigen (in combination with the major histocompatibility complex) as well as adhesion receptors, which promote attachment of
25 T-cells to antigen-presenting cells and transduce signals for T-cell activation. The lymphocyte function associated antigen-1 (LFA-1) has been identified as the major integrin that mediates lymphocyte adhesion and activation leading to a normal immune response, as well as several pathological states (Springer, T.A., Nature 346:425-434 (1990)). Intercellular adhesion molecules (ICAM) -1, -2, and -3, members of the immunoglobulin superfamily, are ligands for LFA-1 found on endothelium,

5 leukocytes and other cell types. The binding of LFA-1 to
ICAMs mediate a range of lymphocyte functions including
lymphokine production of helper T-cells in response to
antigen presenting cells, T-lymphocyte mediated target
cells lysis, natural killing of tumor cells, and
10 immunoglobulin production through T-cell-B-cell
interactions. Thus, many facets of lymphocyte function
involve the interaction of the LFA-1 integrin and its
ICAM ligands. These LFA-1:ICAM mediated interactions
have been directly implicated in numerous inflammatory
15 disease states including; graft rejection, dermatitis,
psoriasis, asthma and rheumatoid arthritis.

20 While LFA-1 (CD11a/CD18) on lymphocytes plays a key role
in chronic inflammation and immune responses, other
members of the leukocyte integrin family (CD11b/CD18,
CD11c/CD18 and CD11d/CD18) also play important roles on
other leukocytes, such as granulocytes and monocytes,
particularly in early response to infective agents and in
acute inflammatory response.

25 The primary function of polymorphonuclear leukocytes,
derived from the neutrophil, eosinophil and basophil
lineage, is to sense inflammatory stimuli and to
emigrate across the endothelial barrier and carry out
30 scavenger function as a first line of host defense. The
integrin Mac-1(CD11b/CD18) is rapidly upregulated on
these cells upon activation and binding to its multiple
ligands which results in the release of oxygen derived
free radicals, protease's and phospholipases. In certain
35 chronic inflammatory states this recruitment is
improperly regulated resulting in significant cellular
and tissue injury. (Harlan, J. M., *Acta Med Scand* ~

5 *Suppl.*, 715:123 (1987); Weiss, S., *New England J. of
Med.*, 320:365 (1989)).

LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18)
The (CD11/CD18) family of adhesion receptor molecules
10 comprises four highly related cell surface glycoproteins;
LFA-1 (CD11a/CD18), Mac-1 (CD11b/CD18), p150.95
(CD11c/CD18) and (CD11d/CD18). LFA-1 is present on the
surface of all mature leukocytes except a subset of
15 macrophages and is considered the major lymphoid
integrin. The expression of Mac-1, p150.95 and
CD11d/CD18 is predominantly confined to cells of the
myeloid lineage (which include neutrophils, monocytes,
macrophage and mast cells). Functional studies have
suggested that LFA-1 interacts with several ligands,
20 including ICAM-1 (Rothlein et al., *J. Immunol.* 137:1270-
1274 (1986), ICAM-2, (Staunton et al., *Nature* 339:361-
364 (1989)), ICAM-3 (Fawcett et al., *Nature* 360:481-484
(1992); Vezeux et al., *Nature* 360:485-488, (1992); de
Fougerolles and Springer, *J. Exp. Med.* 175:185-190
25 (1990)) and Telencephalin (Tian et al., *J. Immunol.*
158:928-936 (1997)).

The CD11/CD18 family is related structurally and
genetically to the larger integrin family of receptors
30 that modulate cell adhesive interactions, which include;
embryogenesis, adhesion to extracellular substrates, and
cell differentiation (Hynes, R. O., *Cell* 48:549-554
(1987); Kishimoto et al., *Adv. Immunol.* 46:149-182 (1989);
Kishimoto et al., *Cell* 48:681-690 (1987); Ruoslahti et al.,
35 *Science* 238:491-497 (1987).

Integrins are a class of membrane-spanning heterodimers
comprising an α subunit in noncovalent association with a

5 β subunit. The β subunits are generally capable of association with more than one α subunit and the heterodimers sharing a common β subunit have been classified as subfamilies within the integrin population (Larson and Springer, "Structure and function of
10 leukocyte integrins," *Immunol. Rev.* 114:181-217 (1990)).

The integrin molecules of the CD11/CD18 family, and their cellular ligands, have been found to mediate a variety of cell-cell interactions, especially in inflammation.
15 These proteins have been demonstrated to be critical for adhesive functions in the immune system (Kishimoto et al., *Adv. Immunol.* 46:149-182 (1989)). Monoclonal antibodies to LFA-1 have been shown to block leukocyte adhesion to endothelial cells (Dustin et al., *J. Cell. Biol.* 107:321-
20 331 (1988); Smith et al., *J. Clin. Invest.* 83:2008-2017 (1989)) and to inhibit T-cell activation (Kuypers et al., *Res. Immunol.*, 140:461 (1989)), conjugate formation required for antigen-specific CTL killing (Kishimoto et al., *Adv. Immunol.* 46:149-182 (1989)), T. cell
25 proliferation (Davignonet et al., *J. Immunol.* 127:590-595 (1981)) and NK cell killing (Krensky et al., *J. Immunol.* 131:611-616 (1983)).

ICAMs

30 ICAM-1 (CD54) is a cell surface adhesion receptor that is a member of the immunoglobulin protein super-family (Rothlein et al., *J. Immunol.* 137:1270-1274 (1986); Staunton et al., *Cell* 52:925-933 (1988). Members of this superfamily are characterized by the presence of one or
35 more Ig homology regions, each consisting of a disulfide-bridged loop that has a number of anti-parallel β -pleated strands arranged in two sheets. Three types of homology

5 regions have been identified, each with a typical length and having a consensus sequence of amino acid residues located between the cysteines of the disulfide bond (Williams, A. F. et al. *Ann Rev. Immunol.* 6:381-405 (1988); Hunkapillar, T. et al. *Adv. Immunol.* 44:1-63 (1989). ICAM-1 is expressed on a variety of hematopoietic and non-hematopoietic cells and is upregulated at sites of inflammation by a variety of inflammatory mediators (Dustin et al., *J. Immunol.*, 137:256-254 (1986)). ICAM-1 is a 90,000-110,000 M_r 10 glycoprotein with a low messenger RNA levels and moderate surface expression on unstimulated endothelial cells. LPS, IL-1 and TNF strongly upregulate ICAM-1 mRNA and surface expression with peak expression at approximately 18-24 hours (Dustinet al., *J. Cell. Biol.* 107:321-331 (1988); Stauntonet al., *Cell* 52:925-933 (1988)). ICAM-1 15 has five extracellular Ig like domains (designated Domains 1, 2, 3, 4 and 5 or D1, D2, D3, D4 and D5) and an intracellular or cytoplasmic domain. The structures and sequence of the domains is described by Staunton et al. (Cell 52:925-933 (1988)).

ICAM-1 was defined originally as a counter-receptor for LFA-1 (Springer et al., *Ann. Rev. Immunol.*, 5:223-252 (1987); Marlin*Cell* 51:813-819 (1987); Simmonset al., 20 *Nature* 331:624-627 (1988); Staunton*Nature* 339:61-64 (1989); Stauntonet al., *Cell* 52:925-933 (1988)). The LFA-1/ICAM-1 interaction is known to be at least partially responsible for lymphocyte adhesion (Dustinet al., *J. Cell. Biol.* 107:321-331 (1988); Mentzeret al., *J. Cell. Physiol.* 126:285-290 (1986)), monocyte adhesion (Amaoutet al., *J. Cell Physiol.* 137:305 (1988); Mentzeret al., *J. Cell. Physiol.* 130:410-415 (1987); te Veldeet al., *Immunology* 61:261-267 (1987)), and neutrophil 25

5 adhesion (Loet al., *J. Immunol.* 143(10):3325-3329 (1989);
Smith et al., *J. Clin. Invest.* 83:2008-2017 (1989)) to
endothelial cells. Through the development of function
blocking monoclonal antibodies to ICAM-1 additional
ligands for LFA-1 were identified, ICAM-2 and ICAM-3
10 (Simmons, *Cancer Surveys* 24, Cell Adhesion and Cancer,
1995) that mediate the adhesion of lymphocytes to other
leukocytes as well as non-hematopoietic cells.
Interactions of LFA-1 with ICAM-2 are thought to mediate
natural killer cell activity (Helander et al., *Nature*
15 382:265-267 (1996)) and ICAM-3 binding is thought to play
a role in lymphocyte activation and the initiation of the
immune response (Simmons, *ibid*). The precise role of
these ligands in normal and aberrant immune responses
remains to be defined.

20

Disorders Mediated by T Lymphocytes

Function blocking monoclonal antibodies have shown that
LFA-1 is important in T-lymphocyte-mediated killing, T-
helper lymphocyte responses, natural killing, and
25 antibody-dependent killing (Springer et al., *Ann. Rev. Immunol.* 5:223-252 (1987)). Adhesion to the target cell
as well as activation and signaling are steps that are
blocked by antibodies against LFA-1.

30

Many disorders and diseases are mediated through T
lymphocytes and treatment of these diseases have been
addressed through many routes. Rheumatoid arthritis
(RA) is one such disorder. Current therapy for RA
includes bed rest, application of heat, and drugs.

35

Salicylate is the currently preferred treatment drug,
particularly as other alternatives such as
immunosuppressive agents and adrenocorticosteroids can
cause greater morbidity than the underlying disease

5 itself. Nonsteroidal anti-inflammatory drugs are available, and many of them have effective analgesic, anti-pyretic and anti-inflammatory activity in RA patients. These include cyclosporin, indomethacin, phenylbutazone, phenylacetic acid derivatives such as ibuprofen and fenoprofen, naphthalene acetic acids (naproxen), pyrrolealkanoic acid (tometin), indoleacetic acids (sulindac), halogenated anthranilic acid (meclofenamate sodium), piroxicam, and diflunisal. Other drugs for use in RA include anti-malarials such as 10 chloroquine, gold salts and penicillamine. These alternatives frequently produce severe side effects, including retinal lesions and kidney and bone marrow toxicity. Immunosuppressive agents such as methotrexate have been used only in the treatment of severe and 15 unremitting RA because of their toxicity. Corticosteroids also are responsible for undesirable side effects (e.g., cataracts, osteoporosis, and Cushing's disease syndrome) and are not well tolerated in many RA patients.

25 Another disorder mediated by T lymphocytes is host rejection of grafts after transplantation. Attempts to prolong the survival of transplanted allografts and xenografts, or to prevent host versus graft rejection, 30 both in experimental models and in medical practice, have centered mainly on the suppression of the immune apparatus of the host/recipient. This treatment has as its aim preventive immunosuppression and/or treatment of graft rejection. Examples of agents used for preventive 35 immunosuppression include cytotoxic drugs, anti-metabolites, corticosteroids, and anti-lymphocytic serum. Nonspecific immunosuppressive agents found particularly effective in preventive immunosuppression

5 (azathioprine, bromocryptine, methylprednisolone,
prednisone, and most recently, cyclosporin A) have
significantly improved the clinical success of
transplantation. The nephrotoxicity of cyclosporin A
after renal transplantation has been reduced by co-
10 administration of steroids such as prednisolone, or
prednisolone in conjunction with azathioprine. In
addition, kidneys have been grafted successfully using
anti-lymphocyte globulin followed by cyclosporin A.
Another protocol being evaluated is total lymphoid
15 irradiation of the recipient prior to transplantation
followed by minimal immunosuppression after
transplantation.

20 Treatment of rejection has involved use of steroids, 2-
amino-6-aryl-5-substituted pyrimidines, heterologous
anti-lymphocyte globulin, and monoclonal antibodies to
various leukocyte populations, including OKT-3. See
generally *J. Pediatrics*, 111: 1004-1007 (1987), and
specifically U.S. Pat. No. 4,665,077.

25 The principal complication of immunosuppressive drugs is
infections. Additionally, systemic immunosuppression is
accompanied by undesirable toxic effects (e.g.,
nephrotoxicity when cyclosporin A is used after renal
30 transplantation) and reduction in the level of the
hemopoietic stem cells. Immunosuppressive drugs may
also lead to obesity, poor wound healing, steroid
hyperglycemia, steroid psychosis, leukopenia,
gastrointestinal bleeding, lymphoma, and hypertension.

35 In view of these complications, transplantation
immunologists have sought methods for suppressing immune
responsiveness in an antigen-specific manner (so that

5 only the response to the donor alloantigen would be
lost). In addition, physicians specializing in
autoimmune disease strive for methods to suppress
autoimmune responsiveness so that only the response to
the self-antigen is lost. Such specific
10 immunosuppression generally has been achieved by
modifying either the antigenicity of the tissue to be
grafted or the specific cells capable of mediating
rejection. In certain instances, whether immunity or
tolerance will be induced depends on the manner in which
15 the antigen is presented to the immune system.

Pretreating the allograft tissues by growth in tissue culture before transplantation has been found in two murine model systems to lead to permanent acceptance
20 across MHC barriers. Lafferty et al., *Transplantation*, 22:138-149 (1976); Bowen et al., *Lancet*, 2:585-586 (1979). It has been hypothesized that such treatment results in the depletion of passenger lymphoid cells and thus the absence of a stimulator cell population
25 necessary for tissue immunogenicity. Lafferty et al., *Annu. Rev. Immunol.*, 1:143 (1983). See also Lafferty et al., *Science*, 188:259-261 (1975) (thyroid held in organ culture), and Gores et al., *J. Immunol.*, 137:1482-1485 (1986) and Faustman et al., *Proc. Natl. Acad. Sci. U.S.A.*, 78: 5156-5159 (1981) (islet cells treated with murine anti-Ia antisera and complement before transplantation). Also, thyroids taken from donor animals pretreated with lymphocytotoxic drugs and gamma radiation and cultured for ten days *in vitro* were not rejected by any normal allogeneic recipient (Gose and Bach, *J. Exp. Med.*, 149:1254-1259 (1979)). All of these techniques involve depletion or removal of donor lymphocyte cells.

5

In some models such as vascular and kidney grafts, there exists a correlation between Class II matching and prolonged allograft survival, a correlation not present in skin grafts (Pescovitz et al., *J.Exp.Med.*, 160:1495-10 1508 (1984); Conti et al., *Transplant. Proc.*, 19: 652-654 (1987)). Therefore, donor-recipient HLA matching has been utilized. Additionally, blood transfusions prior to transplantation have been found to be effective (Opelz et al., *Transplant. Proc.*, 4: 253 (1973); Persijn 15 et al., *Transplant. Proc.*, 23:396 (1979)). The combination of blood transfusion before transplantation, donor-recipient HLA matching, and immunosuppression therapy (cyclosporin A) after transplantation was found to improve significantly the rate of graft survival, and 20 the effects were found to be additive (Opelz et al., *Transplant. Proc.*, 17:2179 (1985)).

The transplantation response may also be modified by antibodies directed at immune receptors for MHC antigens 25 (Bluestone et al., *Immunol. Rev.* 90:5-27 (1986)). Further, graft survival can be prolonged in the presence of antigrift antibodies, which lead to a host reaction that in turn produces specific immunosuppression (Lancaster et al., *Nature*, 315: 336-337 (1985)). The 30 immune response of the host to MHC antigens may be modified specifically by using bone marrow transplantation as a preparative procedure for organ grafting. Thus, anti-T-cell monoclonal antibodies are used to deplete mature T-cells from the donor marrow 35 inoculum to allow bone marrow transplantation without incurring graft-versus-host disease (Mueller-Ruchholtz et al., *Transplant. Proc.*, 8:537-541 (1976)). In addition, elements of the host's lymphoid cells that

5 remain for bone marrow transplantation solve the problem
of immunoincompetence occurring when fully allogeneic
transplants are used.

As shown in Fig. 1, lymphocyte adherence to endothelium
10 is a key event in the process of inflammation. There
are at least three known pathways of lymphocyte
adherence to endothelium, depending on the activation
state of the T-cell and the endothelial cell. T-cell
immune recognition requires the contribution of the T-
15 cell receptor as well as adhesion receptors, which
promote attachment of - cells to antigen-presenting
cells and transduce regulatory signals for T-cell
activation. The lymphocyte function associated (LFA)
antigen-1 (LFA-1, CD11a/CD18, $\alpha_L\beta_2$: where α_L is CD11a
20 and β_2 is CD18) has been identified as the major
integrin receptor on lymphocytes involved in these cell
adherence interactions leading to several pathological
states. ICAM-1, the endothelial cell immunoglobulin-
like adhesion molecule, is a known ligand for LFA-1 and
25 is implicated directly in graft rejection, psoriasis,
and arthritis.

LFA-1 is required for a range of leukocyte functions,
including lymphokine production of helper T-cells in
30 response to antigen-presenting cells, killer T-cell-
mediated target cell lysis, and immunoglobulin
production through T-cell/B-cell interactions.
Activation of antigen receptors on T-cells and B-cells
allows LFA-1 to bind its ligand with higher affinity.

35

Monoclonal antibodies (MAbs) directed against LFA-1 led
to the initial identification and investigation of the

5 function of LFA-1 (Davignon et al., *J. Immunol.*, 127:590
10 (1981)). LFA-1 is present only on leukocytes (Krenskey
et al., *J. Immunol.*, 131:611 (1983)), and ICAM-1 is
distributed on activated leukocytes, dermal fibroblasts,
and endothelium (Dustin et al., *J. Immunol.* 137:245
15 (1986)).

Previous studies have investigated the effects of anti-
CD11a MAbs on many T-cell-dependent immune functions *in*
15 *vitro* and a limited number of immune responses *in vivo*.
In *vitro*, anti-CD11a MAbs inhibit T-cell activation
(Kuypers et al., *Res. Immunol.*, 140:461 (1989)), T-cell-
dependent B-cell proliferation and differentiation
(Davignon et al., *supra*; Fischer et al., *J. Immunol.*,
20 136:3198 (1986)), target cell lysis by cytotoxic T-
lymphocytes (Krenskey et al., *supra*), formation of immune
conjugates (Sanders et al., *J. Immunol.*, 137:2395
(1986); Mentzer et al., *J. Immunol.*, 135:9 (1985)), and
the adhesion of T-cells to vascular endothelium (Lo et
al., *J. Immunol.*, 143:3325 (1989)). Also, the antibody
25 5C6 directed against CD11b/CD18 was found to prevent
intra-islet infiltration by both macrophages and T cells
and to inhibit development of insulin-dependent diabetes
mellitis in mice (Hutchings et al., *Nature*, 348: 639
(1990)).

30 The observation that LFA-1:ICAM-1 interaction is
necessary to optimize T-cell function *in vitro*, and that
anti-CD11a MAbs induce tolerance to protein antigens
(Benjamin et al., *Eur. J. Immunol.*, 18:1079 (1988)) and
35 prolongs tumor graft survival in mice (Heagy et al.,
Transplantation, 37: 520-523 (1984)) was the basis for
testing the MAbs to these molecules for prevention of
graft rejection in humans.

5

Experiments have also been carried out in primates. For example, based on experiments in monkeys it has been suggested that a MAb directed against ICAM-1 can prevent or even reverse kidney graft rejection (Cosimi et al., "Immunosuppression of Cynomolgus Recipients of Renal Allografts by R6.5, a Monoclonal Antibody to Intercellular Adhesion Molecule-1," in Springer et al. (eds.), *Leukocyte Adhesion Molecules* New York: Springer, (1988), p. 274; Cosimi et al., *J. Immunology*, 144:4604-4612 (1990)). Furthermore, the *in vivo* administration of anti-CD11a MAb to cynomolgus monkeys prolonged skin allograft survival (Berlin et al., *Transplantation*, 53: 840-849 (1992)).

The first successful use of a rat anti-murine CD11a antibody (25-3; IgG1) in children with inherited disease to prevent the rejection of bone-marrow-mismatched haploidentical grafts was reported by Fischer et al., *Lancet*, 2: 1058 (1986). Minimal side effects were observed. See also Fischer et al., *Blood*, 77: 249 (1991); van Dijken et al., *Transplantation*, 49:882 (1990); and Perez et al., *Bone Marrow Transplantation*, 4:379 (1989). Furthermore, the antibody 25-3 was effective in controlling steroid-resistant acute graft-versus-host disease in humans. (Stoppa et al., *Transplant. Int.*, 4:3-7 (1991)).

However, these results were not reproducible in leukemic adult grafting with this MAb (Maraninchi et al., *Bone Marrow Transplant*, 4:147-150 (1989)), or with an anti-CD18 MAb, directed against the invariant chain of LFA-1, in another pilot study (Baume et al., *Transplantation*, 47: 472 (1989)). Furthermore, a rat anti-murine CD11a

5 MAb, 25-3, was unable to control the course of acute
rejection in human kidney transplantation (LeMauff et
al., *Transplantation*, 52: 291 (1991)).

10 A review of the use of monoclonal antibodies in human
transplantation is provided by Dantil and Soullou,
Current Opinion in Immunology, 3:740-747 (1991). An
earlier report showed that brief treatment with either
15 anti-LFA-1 or anti-ICAM-1 MAbs minimally prolonged the
survival of primarily vascularized heterotopic heart
allografts in mice (Isobe et al., *Science*, 255:1125
(1992)). However, combined treatment with both MAbs was
required to achieve long-term graft survival in this
model.

20 Independently, it was shown that treatment with anti-
LFA-1 MAb alone potently and effectively prolongs the
survival of heterotopic (ear-pinnae) nonprimarily
vascularized mouse heart grafts using a maximum dose of
25 4 mg/kg/day and treatment once a week after a daily dose
(Nakakura et al., *J. Heart Lung Transplant.*, 11:223
(1992)). Nonprimarily vascularized heart allografts are
more immunogenic and more resistant to prolongation of
survival by MAbs than primarily vascularized heart
allografts (Warren et al., *Transplant. Proc.*, 5:717
30 (1973); Trager et al., *Transplantation*, 47:587 (1989)).
The latter reference discusses treatment with L3T4
antibodies using a high initial dose and a lower
subsequent dose.

35 Another study on treating a sclerosis-type disease in
rodents using similar antibodies to those used by
Nakakura et al., *supra*, is reported by Yednock et al.,
Nature, 356:63-66 (1992). Additional disclosures on the

5 use of anti-LFA-1 antibodies and ICAM-1, ICAM-2, and
ICAM-3 and their antibodies to treat LFA-1-mediated
disorders include WO 91/18011 published 11/28/91, WO
91/16928 published 11/14/91, WO 91/16927 published
11/14/91, Can. Pat. Appln. 2,008,368 published 6/13/91,
10 WO 90/03400, WO 90/15076 published 12/13/90, WO 90/10652
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25 Other disclosures on the use of LFA-1 and ICAM peptide
fragments and antagonists include; U.S. Pat. No.
5,149,780, U.S. Pat. No. 5,288,854, U.S. Pat. No.
5,340,800, U.S. Pat. No. 5,424,399, U.S. Pat. No.
5,470,953, WO 90/03400, WO 90/13316, WO 90/10652, WO
91/19511, WO 92/03473, WO 94/11400, WO 95/28170, JP
30 4193895, EP 314,863, EP 362,526 and EP 362,531.

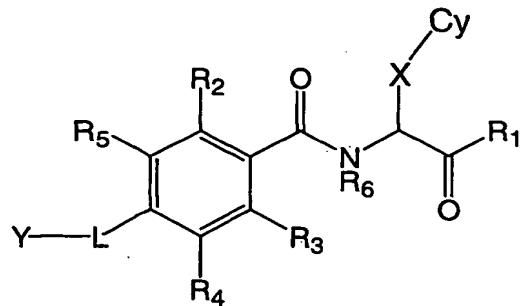
35 The above methods successfully utilizing anti-LFA-1 or
anti-ICAM-1 antibodies, LFA-1 or ICAM-1 peptides,
fragments or peptide antagonists represent an
improvement over traditional immunosuppressive drug
therapy. These studies demonstrate that LFA-1 and ICAM-
1 are appropriate targets for antagonism. There is a
need in the art to better treat disorders that are

5 mediated by LFA-1 including autoimmune diseases, graft
vs. host or host vs. graft rejection, and T-cell
inflammatory responses, so as to minimize side effects
and sustain specific tolerance to self- or xenoantigens.
There is also a need in the art to provide a non-peptide
10 antagonists to the LFA-1: ICAM-1 interaction.

Albumin is an abundant plasma protein which is
responsible for the transport of fatty acids. However,
15 albumin also binds and perturbs the pharmacokinetics of a
wide range of drug compounds. Accordingly, a significant
factor in the pharmacological profile of any drug is its
binding characteristics with respect to serum plasma
proteins such as albumin. A drug compound may have such
20 great affinity for plasma proteins that it is not be
available in serum to interact with its target tissue,
cell or protein. For example, a compound for which 99%
binds to plasma protein upon administration will have
half the concentration available in plasma to interact
25 with its target than a compound which binds only 98%.
Accordingly it would be desirable to provide LFA
antagonist compounds which have low serum plasma protein
binding affinity.

30 SUMMARY OF THE INVENTION

In an aspect of the present invention, there is provided
novel compounds of formula (I)



5

wherein

Cy is a non-aromatic carbocycle or heterocycle optionally substituted with hydroxyl, mercapto, thioalkyl, halogen, oxo, thio, amino, aminoalkyl, amidine, guanidine, nitro, alkyl, alkoxy or acyl;

X is a divalent hydrocarbon chain optionally substituted with hydroxyl, mercapto, halogen, amino, aminoalkyl, nitro, oxo or thio and optionally interrupted with N, O, S, SO or SO₂;

15 Y is a carbocycle or heterocycle optionally substituted with hydroxyl, mercapto, halogen, oxo, thio, a hydrocarbon, a halo-substituted hydrocarbon, amino, amidine, guanidine, cyano, nitro, alkoxy or acyl;

L is a bond or a divalent hydrocarbon optionally having 20 one or more carbon atoms replaced with N, O, S, SO or SO₂ and optionally being substituted with hydroxyl, halogen oxo or thio; or three carbon atoms of the hydrocarbon are replaced with an amino acid residue;

R₁ is H, OH, amino, O-carbocycle or alkoxy optionally 25 substituted with amino, a carbocycle or a heterocycle;

R₂₋₅ are independently H, hydroxyl, mercapto, halogen, cyano, amino, amidine, guanidine, nitro or alkoxy; or

R₃ and R₄ together form a fused carbocycle or 30 heterocycle optionally substituted with hydroxyl, halogen, oxo, thio, amino, amidine, guanidine or alkoxy;

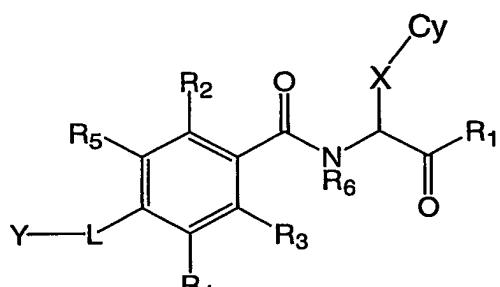
5 R₆ is H or a hydrocarbon chain optionally substituted with
a carbocycle or a heterocycle; and
salts, solvates and hydrates thereof;
with the proviso that when Y is phenyl, R₂, R₄ and R₅ are
H, R₃ is Cl and R₁ is OH then X is other than cyclohexyl.

10 In another aspect of the invention, there is provided
pharmaceutical compositions comprising a compound of the
invention and a pharmaceutically acceptable carrier.

15 In another aspect of the invention, there is provided a
method of treating a disease or condition mediated by
LFA-1 in a mammal comprising administering to said mammal
an effective amount of a compound of the invention.

20 DETAILED DESCRIPTION OF THE INVENTION

The invention provides novel compounds of formula (I)



25 (I)

wherein Cy, X, Y, L and R₁₋₆ are as defined herein.
Compounds of the invention exhibit reduced plasma protein
binding affinity by virtue of a non-aromatic ring at
30 substituent Cy in comparison to those having an aromatic
ring at this portion of the molecule.

5 The term "non-aromatic" refers to carbocycle or heterocycle rings that do not have the properties which define aromaticity. For aromaticity, a ring must be planar, have p-orbitals that are perpendicular to the plane of the ring at each ring atom and satisfy the
10 Huckel rule where the number of pi electrons in the ring is $(4n+2)$ wherein n is an integer (i.e. the number of pi electrons is 2, 6, 10 or 14). Non-aromatic rings provided herein do not satisfy one or all of these criteria for aromaticity.

15 The term "alkoxy" as used herein includes saturated, i.e. O-alkyl, and unsaturated, i.e. O-alkenyl and O-alkynyl, group. Exemplary alkoxy groups include methoxy, ethoxy, propoxy, butoxy, i-butoxy, s-butoxy, t-butoxy, pentyloxy
20 and hexyloxy.

25 The term "amino" refers to a primary ($-NH_2$), secondary ($-NHR$), tertiary ($-N(R)_2$) or quaternary ($-N^+(R)_4$) amine wherein R is a hydrocarbon chain, hydroxy, a carbocycle, a heterocycle or a hydrocarbon chain substituted with a carbocycle or heterocycle.

30 The term "amino acid" refers to naturally and non-naturally occurring α -(alpha), β -(beta), D- and L-amino acid residues. Non-natural amino acids include those having side chains other than those occurring in nature.

35 By "carboxyl" is meant herein to be a free acid $-COOH$ as well as esters thereof such as alkyl, aryl and aralkyl esters. Preferred esters are methyl, ethyl, propyl, butyl, i-butyl, s-butyl and t-butyl esters.

5 The term "carbocycle" refers to a mono-, bi- or tri-cyclic carbon ring or ring system having 4-16 members (including bridged) which is saturated, unsaturated or partially unsaturated including aromatic (aryl) ring systems (unless specified as non-aromatic). Preferred
10 non-aromatic carbocyclic rings include cyclopropyl, cyclopropenyl, cyclobutyl, cyclobutenyl, cyclopentyl, cyclopentenyl, cyclohexyl and cyclohexenyl. Preferred aromatic carbocyclic rings include phenyl and naphthyl.

15 The term "heterocycle" refers to a mono-, bi- or tri-cyclic ring system having 5-16 members wherein at least one ring atom is a heteroatom (i.e. N, O and S as well as SO, or SO₂). The ring system is saturated, unsaturated or partially unsaturated and may be aromatic (unless
20 specified as non-aromatic). Exemplary heterocycles include piperidine, piperazine, pyridine, pyrazine, pyrimidine, pyridazine, morpholine, pyran, pyrole, furan, thiophene (thienyl), imidazole, pyrazole, thiazole, isothiazole, dithiazole, oxazole, isoxazole, dioxazole,
25 thiadiazole, oxadiazole, tetrazole, triazole, thiatriazole, oxatriazole, thiadiazole, oxadiazole, purine and benzofused derivatives thereof.

30 The term "hydrocarbon chain" refers to saturated, unsaturated, linear or branched carbon chains i.e. alkyl, alkenyl and alkynyl. Preferred hydrocarbon chains incorporate 1-12 carbon atoms, more preferably 1-6 and most preferably 1-4 carbon atoms i.e. methyl, ethyl, propyl, butyl and allyl.

35

The phrase "optionally substituted with" is understood to mean, unless otherwise stated, that one or more of the specified substituents is covalently attached to the

5 substituted moiety. When more than one, the substituents
may be the same or different group.

Cy is a non-aromatic carbocycle or heterocycle optionally substituted with hydroxyl (-OH), mercapto (-SH), thioalkyl, halogen (e.g. F, Cl, Br, I), oxo (=O), thio (=S), amino, aminoalkyl, amidine (-C(NH)-NH₂), guanidine (-NH₂-C(NH)-NH₂), nitro, alkyl or alkoxy. In a particular embodiment, Cy is a 3-5 member ring. In a preferred embodiment, Cy is a 5- or 6-member non-aromatic heterocycle optionally substituted with hydroxyl, mercapto, halogen (preferably F or Cl), oxo (=O), thio (=S), amino, amidine, guanidine, nitro, alkyl or alkoxy. In a more preferred embodiment, Cy is a 5-member non-aromatic heterocycle optionally substituted with hydroxyl, oxo, thio, Cl, C₁₋₄ alkyl (preferably methyl), or C₁₋₄ alkanoyl (preferably acetyl, propanoyl or butanoyl). More preferably the non-aromatic heterocycle comprises one or heteroatoms (N, O or S) and is optionally substituted with hydroxyl, oxo, mercapto, thio, methyl, acetyl, propanoyl or butyl. In particular embodiments the non-aromatic heterocycle comprises at least one nitrogen atom that is optionally substituted with methyl or acetyl. In a particularly preferred embodiment, the non-aromatic heterocycle is selected from the group consisting of piperidine, piperazine, morpholine, tetrahydrofuran, tetrahydrothiophene, oxazolidine, thiazolidine optionally substituted with hydroxy, oxo, mercapto, thio, alkyl or alkanoyl. In a most preferred embodiment Cy is a non-aromatic heterocycle selected from the group consisting of tetrahydrofuran-2-yl, thiazolidin-5-yl, thiazolidin-2-one-5-yl, and thiazolidin-2-thione-5-yl and cyclopropapyrrolidine.

5

In another preferred embodiment Cy is a 3-6 member carbocycle optionally substituted with hydroxyl, mercapto, halogen, oxo, thio, amino, amidine, guanidine, alkyl, alkoxy or acyl. In a particular embodiment the carbocycle is saturated or partially unsaturated. In particular embodiments Cy is a carbocycle selected from the group consisting of cyclopropyl, cyclopropenyl, cyclobutyl, cyclobutenyl, cyclopentyl, cyclopentenyl, cyclohexyl and cyclohexenyl.

15

X is a C₁₋₅ divalent hydrocarbon linker optionally having one or more carbon atoms replaced with N, O, S, SO or SO₂ and optionally being substituted with hydroxyl, mercapto, halogen, amino, aminoalkyl, nitro, oxo or thio. In a preferred embodiment X will have at least one carbon atom. Replacements and substitutions may form an amide moiety (-NRC(O)- or -C(O)NR-) within the hydrocarbon chain or at either or both ends. Other moieties include sulfonamide (-NRSO₂- or -SO₂NR), acyl, ether, thioether and amine. In a particularly preferred embodiment X is the group -CH₂-NR₆-C(O)- wherein the carbonyl -C(O)- portion thereof is adjacent (i.e. covalently bound) to Cy and R₆ is alkyl i.e. methyl and more preferably H.

20

Y is a carbocycle or heterocycle optionally substituted with hydroxyl, mercapto, halogen, oxo, thio, a hydrocarbon, a halo-substituted hydrocarbon, amino, amidine, guanidine, cyano, nitro, alkoxy or acyl. In a particular embodiment, Y is aryl or heteroaryl optionally substituted with halogen or hydroxyl. In a particularly preferred embodiment, Y is phenyl, furan-2-yl, thiophene-

5 2-yl, phenyl substituted with a halogen (preferably Cl) or hydroxyl, preferably at the meta position.

L is a divalent hydrocarbon optionally having one or more carbon atoms replaced with N, O, S, SO or SO₂ and 10 optionally being substituted with hydroxyl, halogen oxo, or thio; or three carbon atoms of the hydrocarbon are replaced with an amino acid residue. Preferably L is less than 10 atoms in length and more preferably 5 or less and most preferably 5 or 3 atoms in length. In 15 particular embodiments, L is selected from the group consisting of -CH=CH-C(O)-NR₆-CH₂-, -CH₂-NR₆-C(O)-, -C(O)-NR₆-CH₂-, -CH(OH)-(CH₂)₂-, -(CH₂)₂-CH(OH)-, -(CH₂)₃-, -C(O)-NR₆-CH(R₇)-C(O)-NR₆-, -NR₆-C(O)-CH(R₇)-NR₆-C(O)-, -CH(OH)-CH₂-O- and -CH(OH)-CF₂-CH₂- wherein each R₆ is 20 independently H or alkyl and R₇ is an amino acid side chain. Preferred amino acid side chains include non-naturally occurring side chains such as phenyl or naturally occurring side chains. Preferred side chains are those from Phe, Tyr, Ala, Gln and Asn. In a 25 preferred embodiment L is -CH=CH-C(O)-NR₆-CH₂- wherein the -CH=CH- moiety thereof is adjacent (i.e. covalently bound) to Y. In another preferred embodiment, L is -CH₂-NR₆-C(O)- wherein the methylene moiety (-CH₂-) thereof is adjacent to Y.

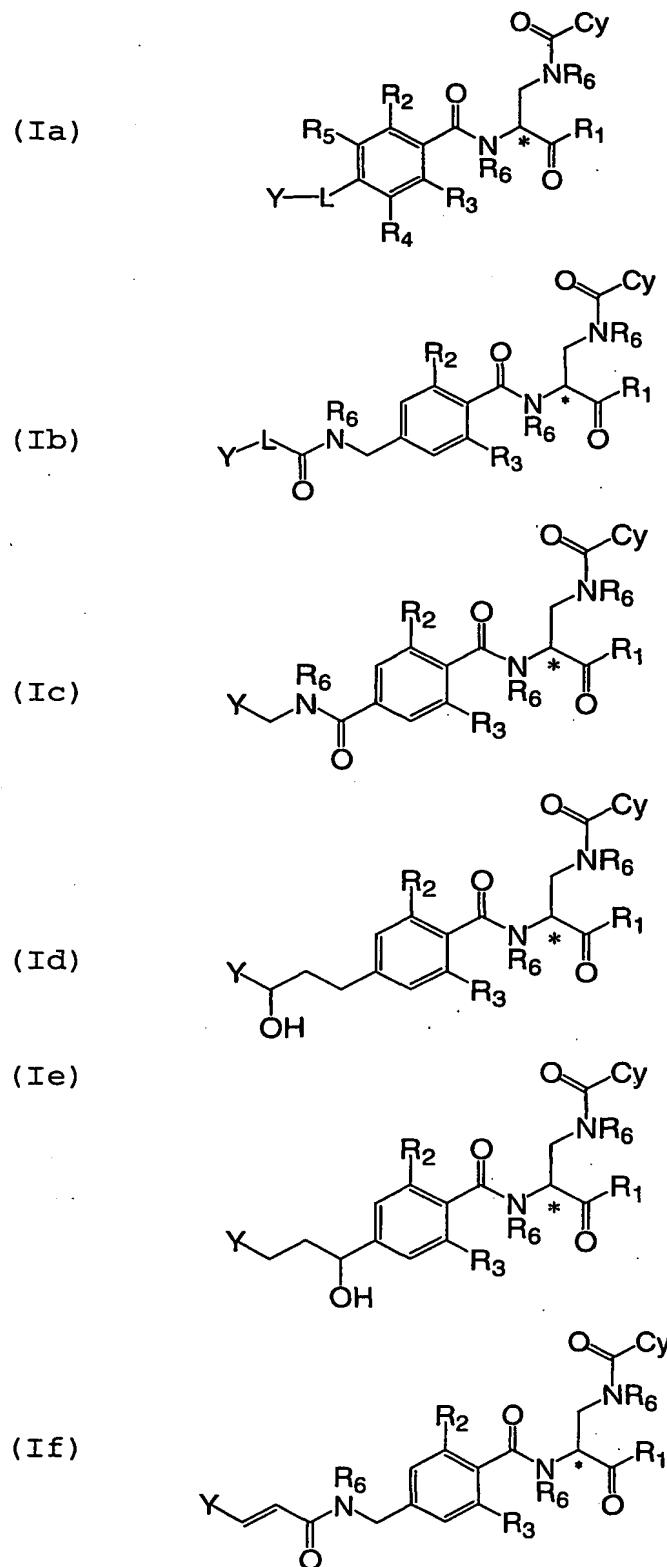
30 R₁ is H, OH, amino, O-carbocycle or alkoxy optionally substituted with amino, a carbocycle or a heterocycle. In a preferred embodiment, R₁ is H, phenyl or C₁₋₄ alkoxy optionally substituted with a carbocycle such as phenyl. 35 In a particular embodiment R₁ is H. In another particular embodiment R₁ is methoxy, ethoxy, propyloxy, butyloxy, isobutyloxy, s-butyloxy, t-butyloxy, phenoxy or benzyloxy. In yet another particular embodiment R₁ is

5 NH₂. In a particularly preferred embodiment R₁ is ethoxy.
In another particularly preferred embodiment R₁ is
isobutyloxy. In another particularly preferred
embodiment R₁ is alkoxy substituted with amino, for
example 2-aminoethoxy, N-morpholinoethoxy, N,N-
10 dialkyaminoethoxy, quaternary ammonium hydroxy alkoxy
(e.g. trimethylammoniumhydroxyethoxy).

R₂₋₅ are independently H, hydroxyl, mercapto, halogen,
cyano, amino, amidine, guanidine, nitro or alkoxy; or R₃
15 and R₄ together form a fused carbocycle or heterocycle
optionally substituted with hydroxyl, halogen, oxo, thio,
amino, amidine, guanidine or alkoxy. In a particular
embodiment R₂ and R₃ are independently H, F, Cl, Br or I.
In another particular embodiment, R₄ and R₅ are both H.
20 In another particular embodiment, one of R₂ and R₃ is a
halogen while the other is hydrogen or a halogen. In a
particularly preferred embodiment, R₃ is Cl while R₂, R₄
and R₅ are each H. In another particularly preferred
embodiment, R₂ and R₃ are both Cl while R₄ and R₅ are both
25 H.

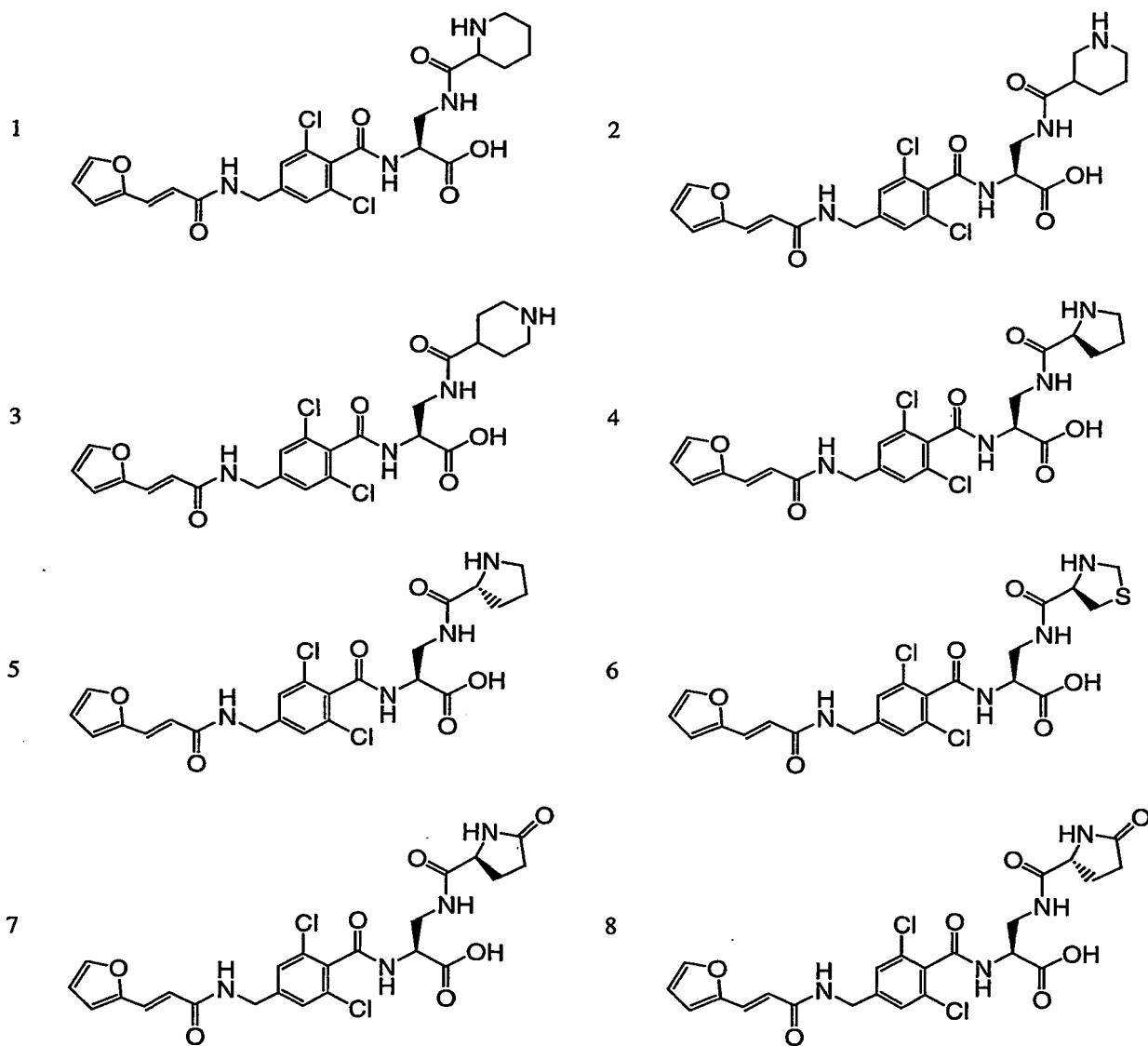
R₆ is H or a hydrocarbon chain optionally substituted with
a carbocycle or a heterocycle. In a preferred
embodiment, R₆ is H or alkyl i.e. methyl, ethyl, propyl,
30 butyl, i-butyl, s-butyl or t-butyl. In a particular
embodiment R₆ is H.

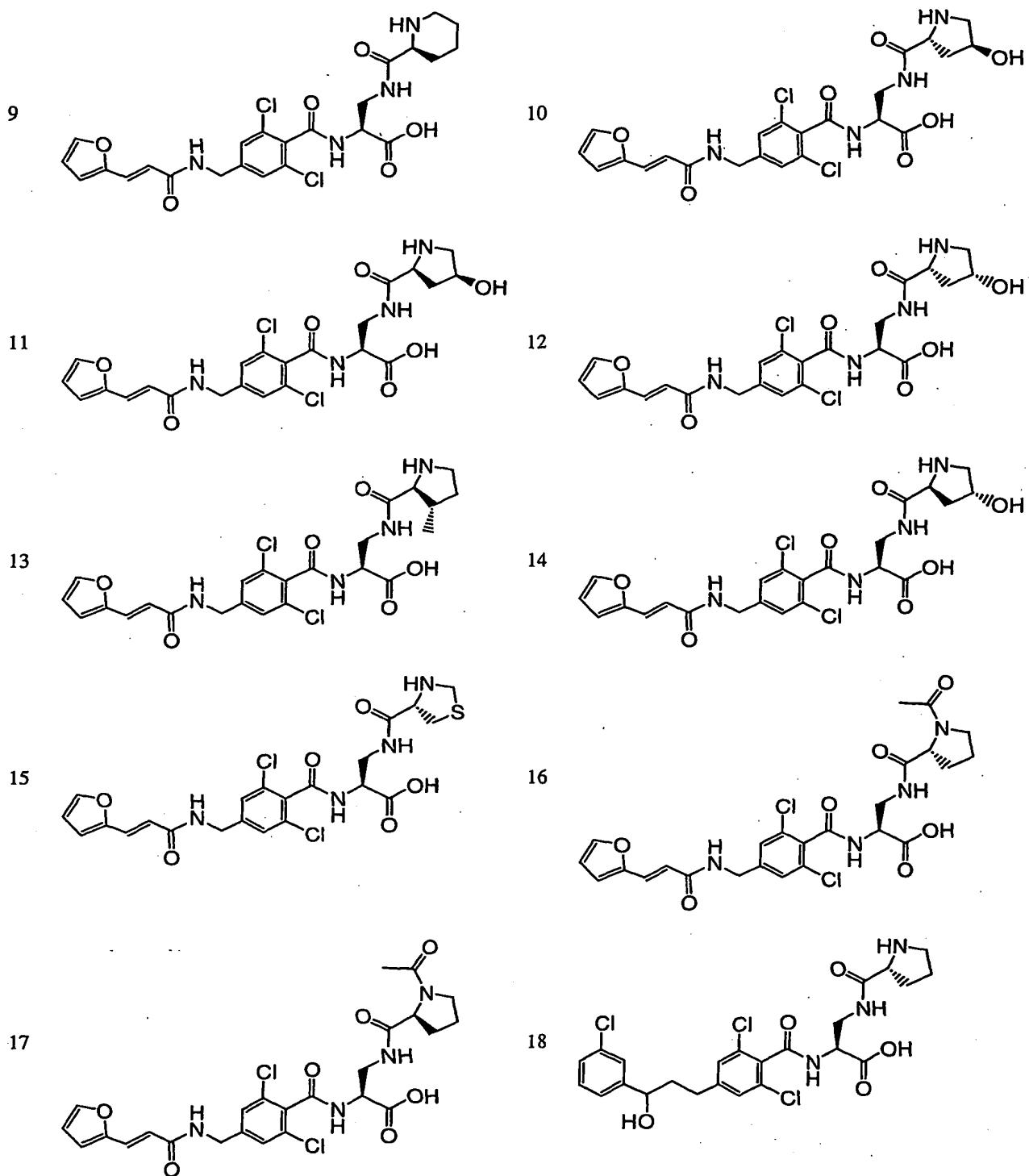
In a preferred embodiment, compounds of the invention
have the general formula (Ia) - (If)

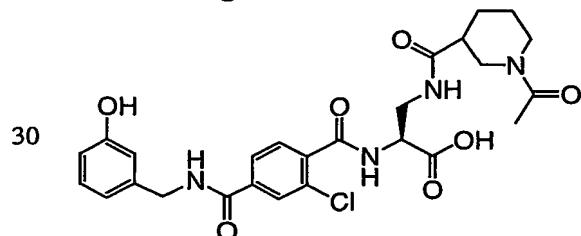
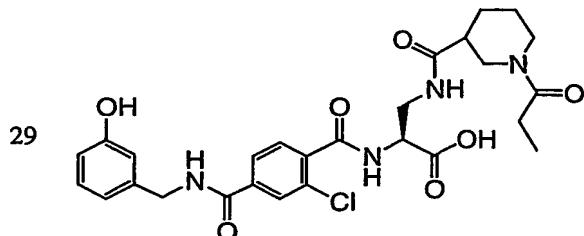
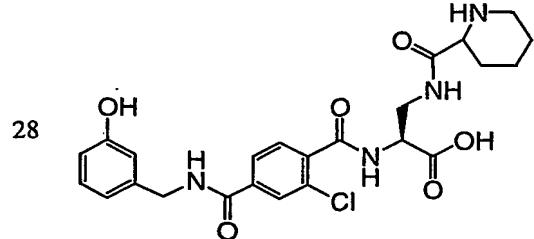
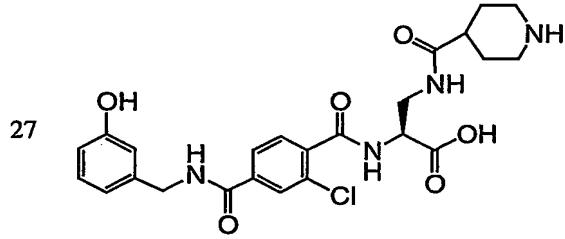
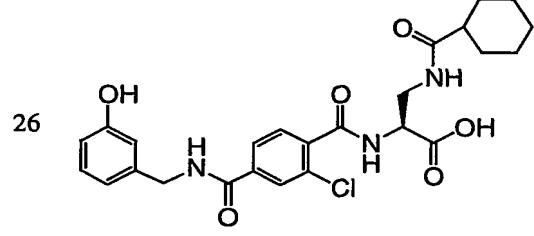
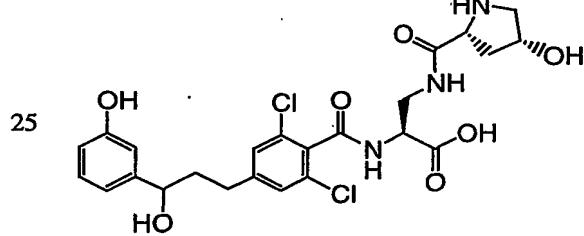
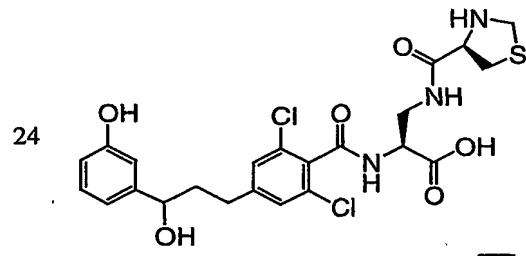
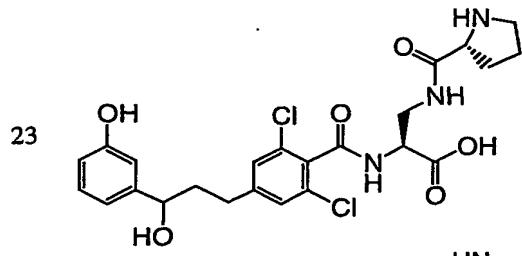
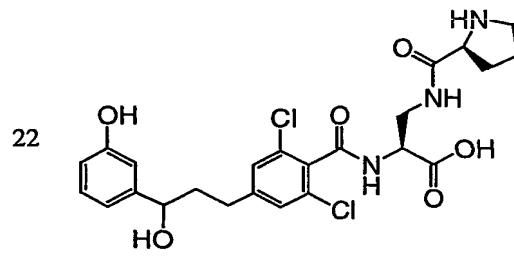
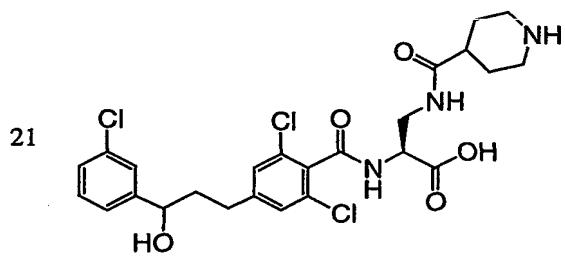
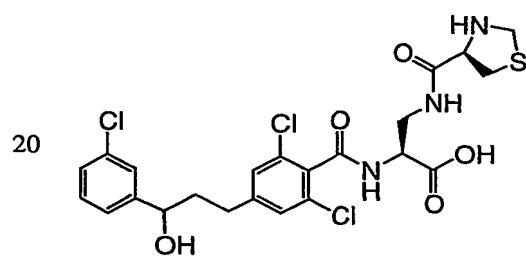
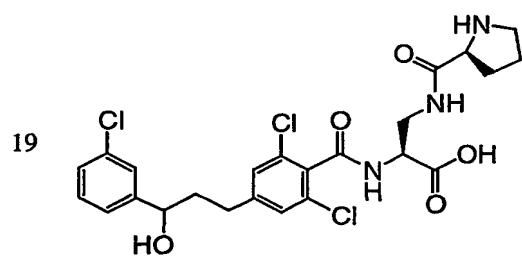


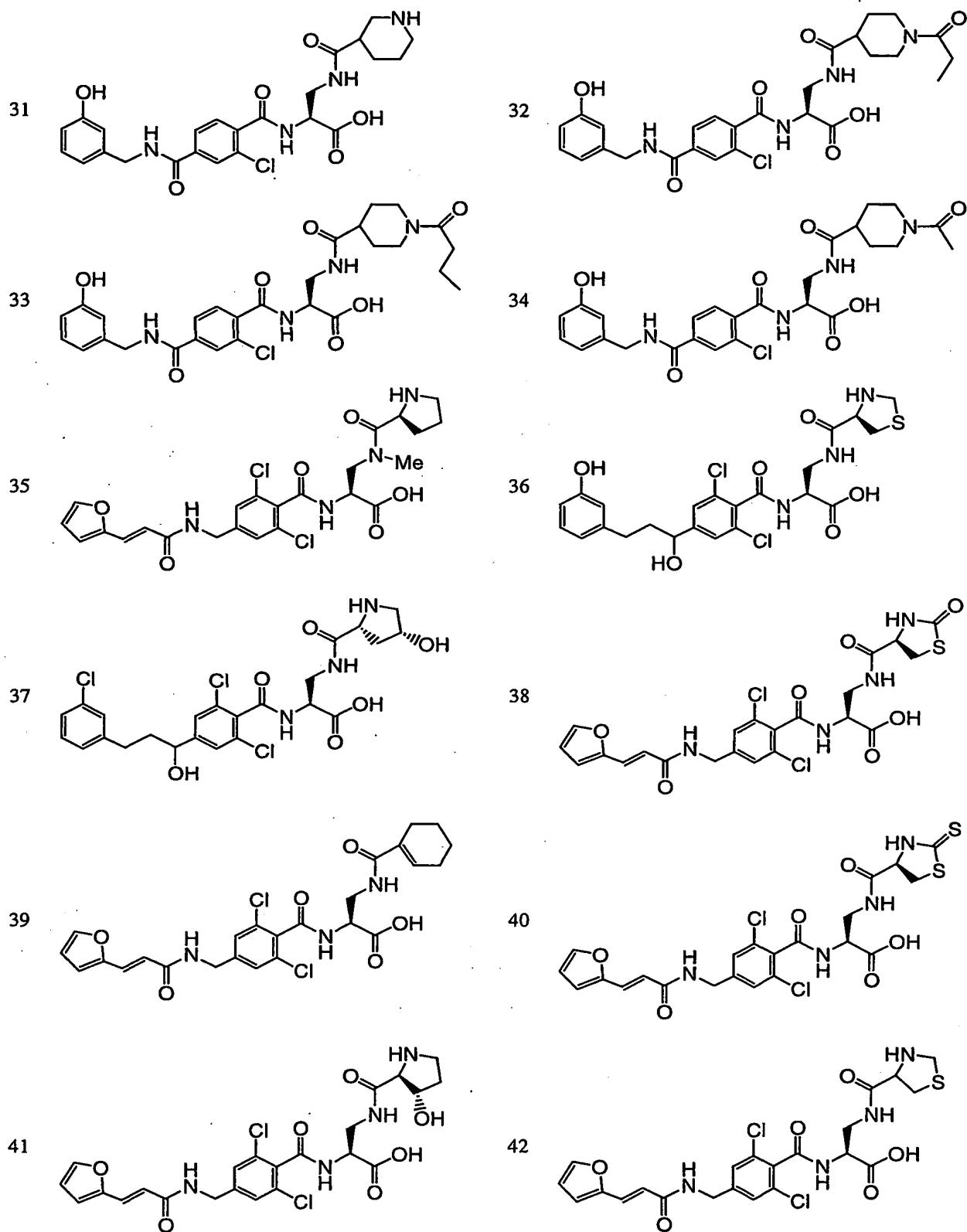
5 wherein Cy, Y, L and R₁₋₆ are as previously defined. In a particularly preferred embodiment, the carbon atom marked with an asterisk (*) in compounds of formula (Ia) - (If) is chiral. In a particular embodiment, the carbon atom has an R-configuration. In another particular
10 embodiment, the carbon atom has an S-configuration.

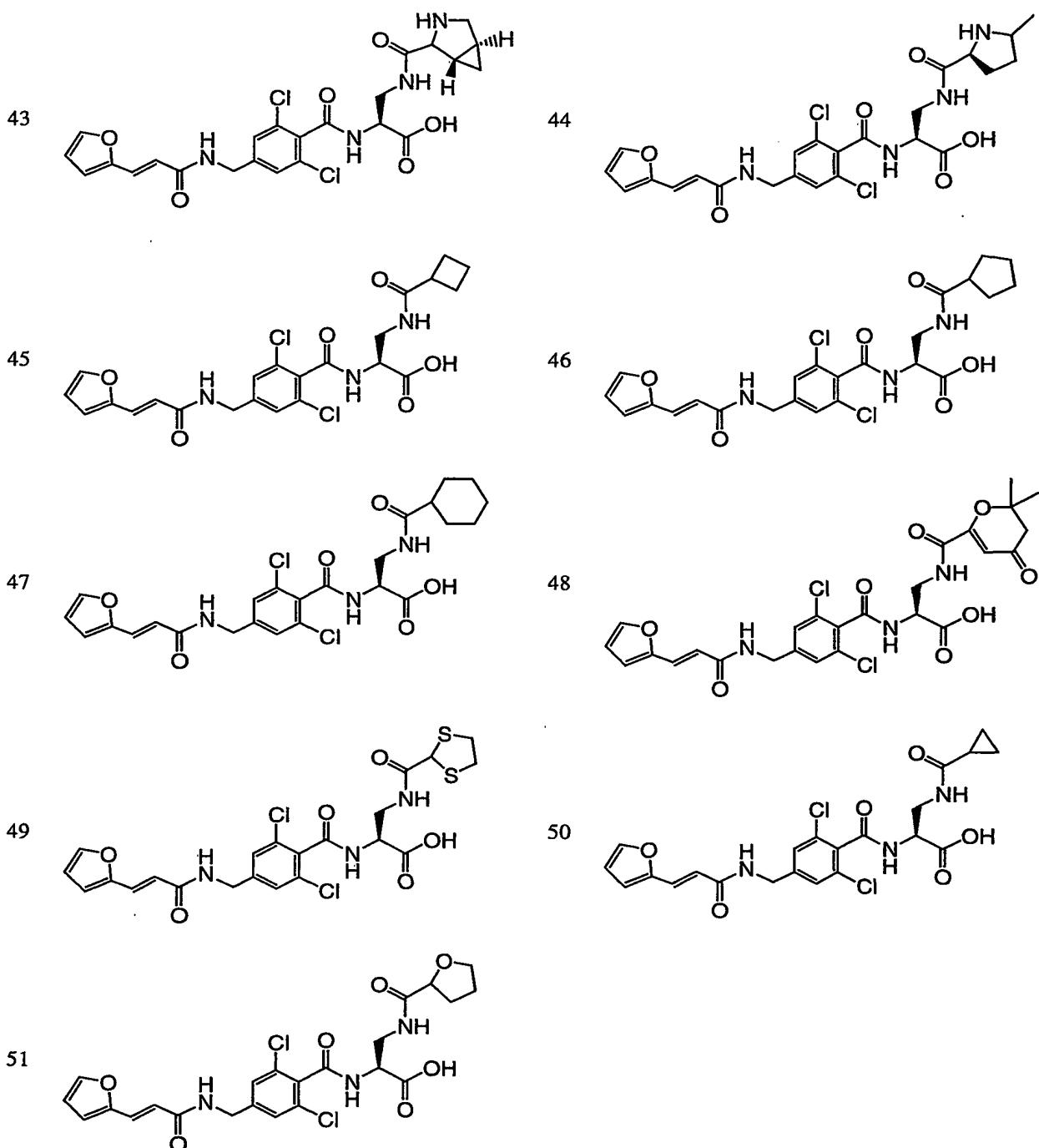
Particular compounds of the invention include:











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and salts, solvates, hydrates and esters thereof.

It will be appreciated that compounds of the invention
10 may incorporate chiral centers and therefore exist as
geometric and stereoisomers. All such isomers are

5 contemplated and are within the scope of the invention whether in pure isomeric form or in mixtures of such isomers as well as racemates. Stereoisomeric compounds may be separated by established techniques in the art such as chromatography, i.e. chiral HPLC, or
10 crystallization methods.

"Pharmaceutically acceptable" salts include both acid and base addition salts. Pharmaceutically acceptable acid addition salt refers to those salts which retain
15 the biological effectiveness and properties of the free bases and which are not biologically or otherwise undesirable, formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, carbonic acid, phosphoric acid and the
20 like, and organic acids may be selected from aliphatic, cycloaliphatic, aromatic, arylaliphatic, heterocyclic, carboxylic, and sulfonic classes of organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, gluconic acid, lactic acid, pyruvic acid, oxalic acid, malic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, aspartic acid, ascorbic acid, glutamic acid, anthranilic acid, benzoic acid, cinnamic acid, mandelic acid, embonic acid, phenylacetic acid, methanesulfonic acid,
25 ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid and the like.
30

Pharmaceutically acceptable base addition salts include those derived from inorganic bases such as sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, aluminum salts and the like. Particularly preferred are the ammonium, potassium, sodium, calcium and magnesium salts. Salts derived from
35

5 pharmaceutically acceptable organic nontoxic bases includes salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as isopropylamine, trimethylamine,
10 diethylamine, triethylamine, tripropylamine, ethanolamine, 2-diethylaminoethanol, trimethamine, dicyclohexylamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, methylglucamine,
15 theobromine, purines, piperazine, piperidine, N-ethylpiperidine, polyamine resins and the like. Particularly preferred organic non-toxic bases are isopropylamine, diethylamine, ethanolamine, trimethamine, dicyclohexylamine, choline, and caffeine.

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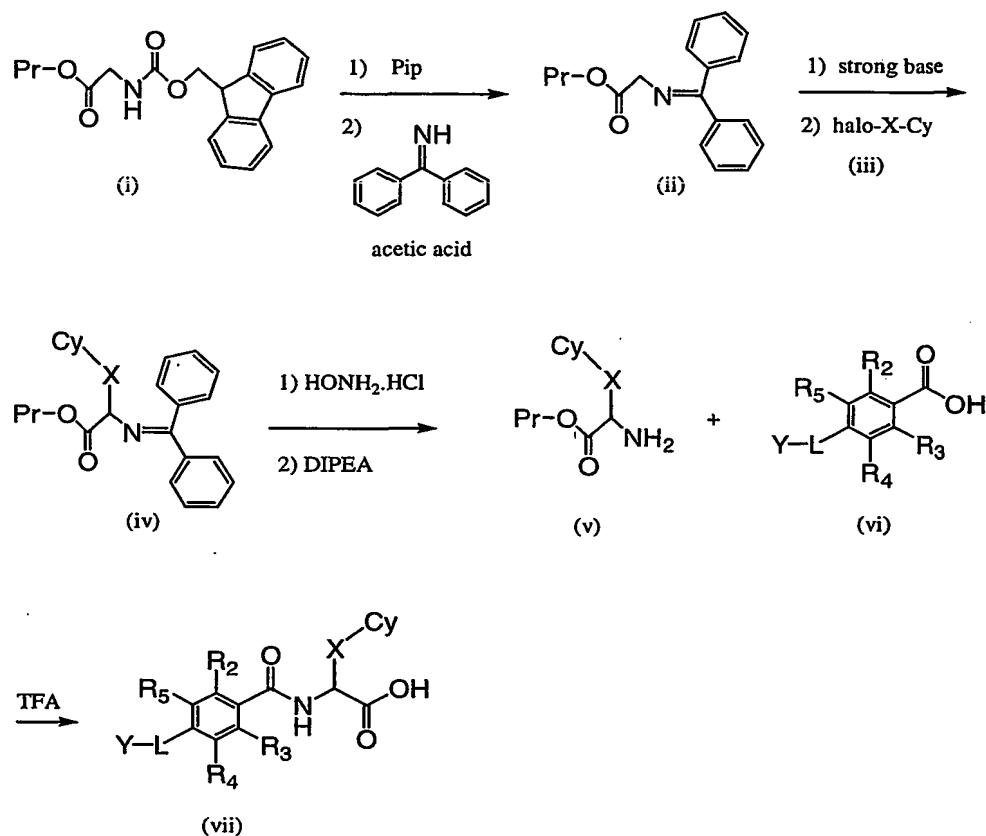
Compounds of the invention may be prepared according to established organic synthesis techniques from starting materials and reagents that are commercially available or
25 from starting materials that may be prepared from commercially available starting materials. Many standard chemical techniques and procedures are described in March, J., "Advanced Organic Chemistry" McGraw-Hill, New York, 1977; and Collman, J., "Principles and Applications
30 of Organotransition Metal Chemistry" University Science, Mill Valley, 1987; and Larock, R., "Comprehensive Organic Transformations" Verlag, New York, 1989. It will be appreciated that depending on the particular substituents present on the compounds, suitable protection and
35 deprotection procedures will be required in addition to those steps described herein. Numerous protecting groups are described in Greene and Wuts, Protective Groups in Organic Chemistry, 2d edition, John Wiley and Sons, 1991,

5 as well as detailed protection and deprotection procedures. For example, suitable amino protecting groups include t-butyloxycarbonyl (Boc), fluorenyl-methyloxycarbonyl (Fmoc), 2-trimethylsilyl-ethyoxy-carbonyl (Teoc), 1-methyl-1-(4-biphenyl)ethoxycarbonyl (Bpoc), allyloxycarbonyl (Alloc), and benzyloxycarbonyl (Cbz). Carboxyl groups can be protected as fluorenyl-methyl groups, or alkyl esters i.e. methyl or ethyl, or alkenyl esters such as allyl. Hydroxyl groups may be protected with trityl, monomethoxytrityl, dimethoxytrityl, and trimethoxytrityl groups.

Compounds may be prepared according to organic synthetic procedures described in United States patent application 09/6446,330 filed on 14 September 2000, the entirety of which is incorporated herein by reference. Generally, compounds may be prepared according to reaction scheme 1.

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Scheme 1



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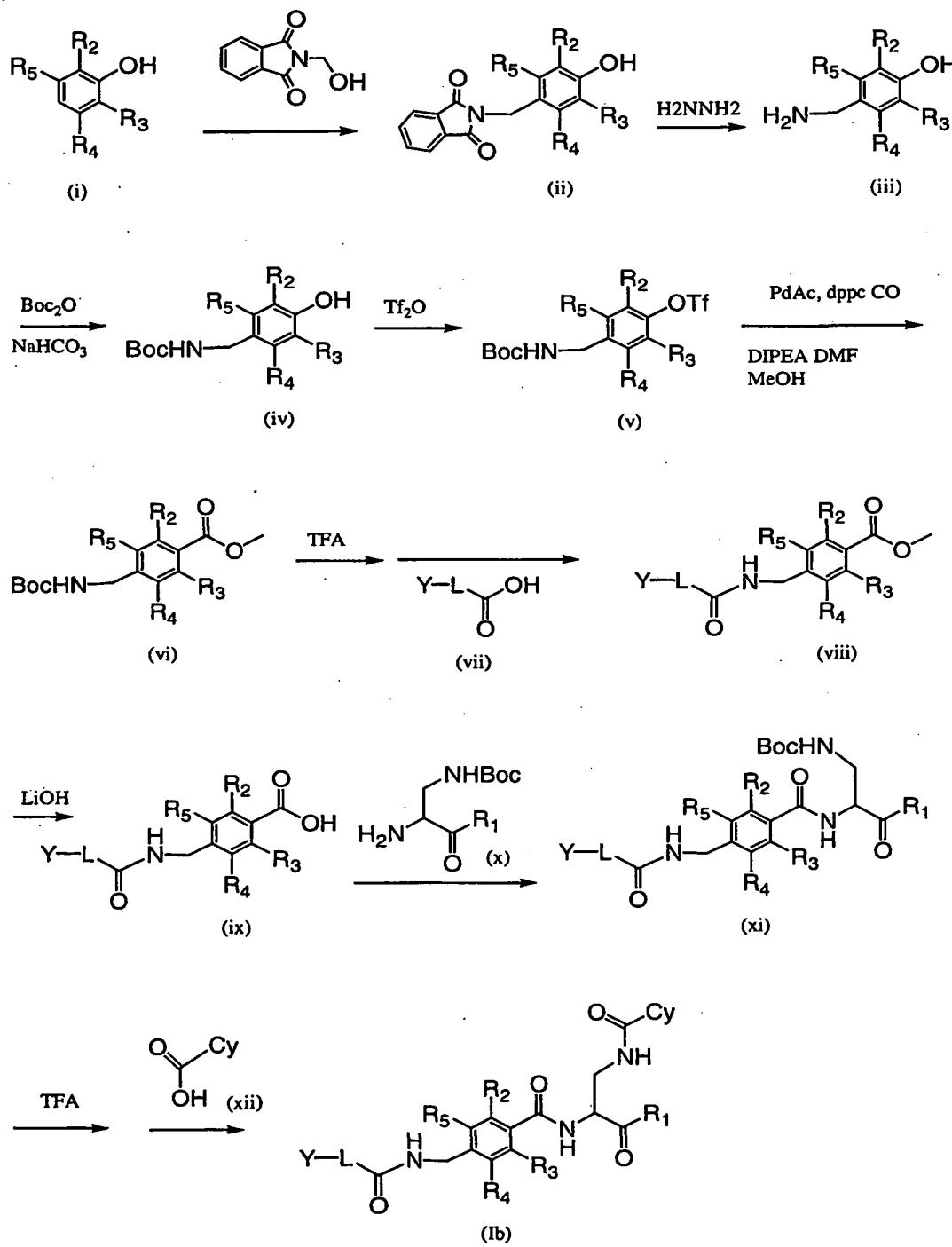
Referring to scheme 1, a commercially available glycine amino acid residue is protected at the amino (e.g. fmoc) and carboxyl groups (Pr) or else immobilized on a solid support. The amino protecting group is removed with a suitable reagent and is reacted with diphenylketimine and subsequently alkylated at the alpha carbon with (iii) halo-X-Cy to give intermediate (vi). The imine (vi) is converted to the free amine (v) and then coupled with intermediate (vi) to provide the compound of the invention which is optionally deprotected at the carboxyl group to give free acid (vii). The free acid in turn may be esterified or amidated according to the definitions of substituent R₁.

5

In a particular embodiment, compounds of formula (Ib) of the invention may be prepared according to scheme 2.

Scheme 2

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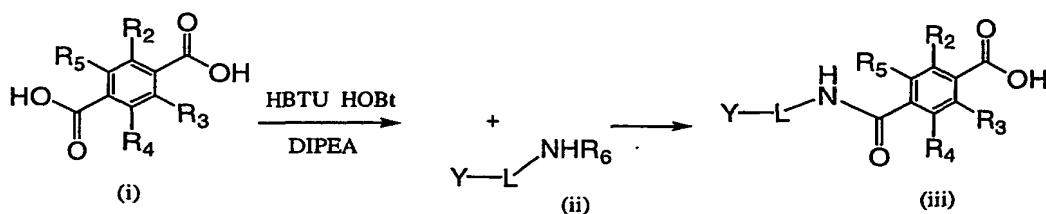


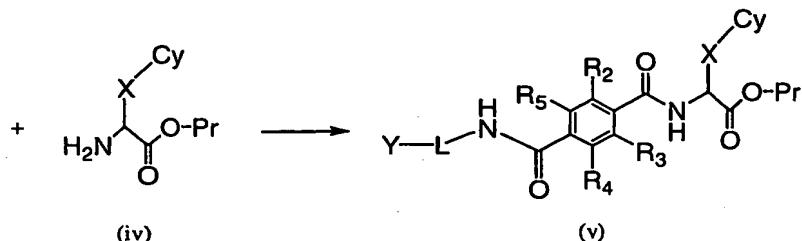
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5 Referring to scheme 2, starting compound (i), commercially available or synthesized from commercially available reagents, is reacted with N-hydroxymethylphthalimide to give intermediate (ii) which is reacted with hydrazine to yield the free amine (iii).
 10 The amine is Boc protected (iv) by reacting with Boc₂O and sodium bicarbonate and then reacted with triflic anhydride to give intermediate (v). The triflate intermediate (v) is then converted to the methyl ester intermediate (vi) by reacting with palladium(II) acetate and 1,3-
 15 bi(diphenylphosphino)propane (dppp) and subsequently with diisopropyl ethylamine (DIPEA). The Boc group of (vi) is removed with TFA and then reacted with carboxylic acid (vii) to give intermediate (viii). In a preferred embodiment of scheme 2, intermediate (vii) Y-L-C(O)OH is
 20 furylacrylic acid or thienylacrylic acid. The methyl ester of (viii) is removed with LiOH to give the free acid which is reacted with the N-Boc protected diaminopropanoic acid/ester (x) to yield intermediate (xi). The Boc group of (xi) is removed with TFA and then reacted with carboxyl-substituted non-aromatic ring (xii)
 25 to give final compound (Ib) of the invention.

In another particular embodiment compounds of formula (Ic) of the invention may be prepared according to scheme 30 3.

Scheme 3





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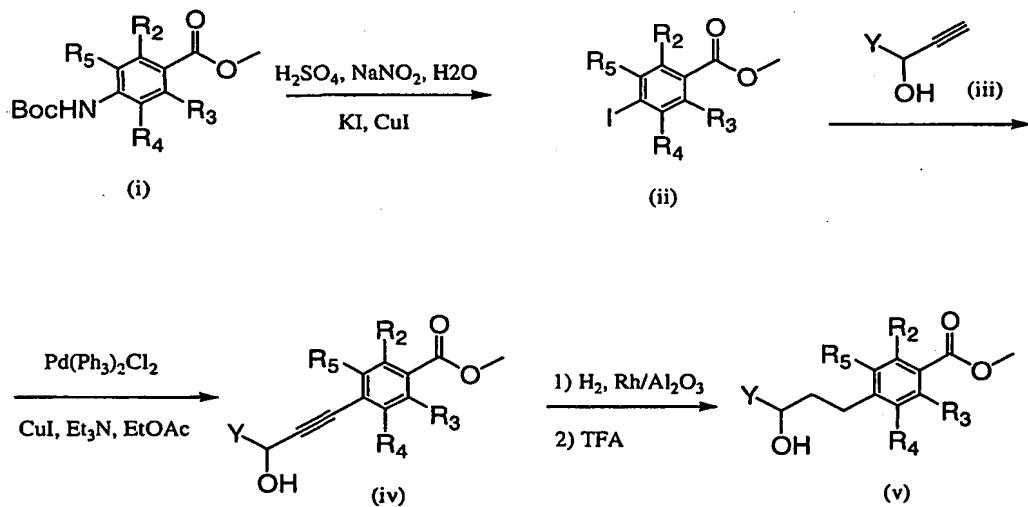
Referring to scheme 3, carboxylate starting reagent (i) is coupled with amine reagent (ii) $Y-L-NHR_6$ to give intermediate (iii) which is coupled with (iv) to yield compound of the invention (v). In a preferred embodiment of scheme 3, $Y-L-$ is benzyl, optionally substituted with hydroxy, halogen, alkyl or alkoxy. More preferably $Y-L-$ is 3-hydroxy-benzyl.

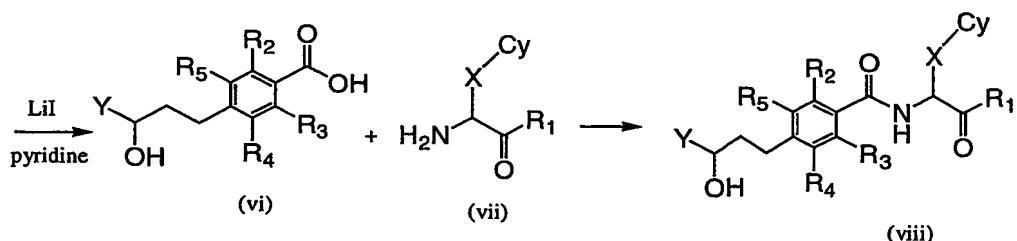
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In another particular embodiment, compounds of formula (Id) of the invention may be prepared according to scheme 4.

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Scheme 4



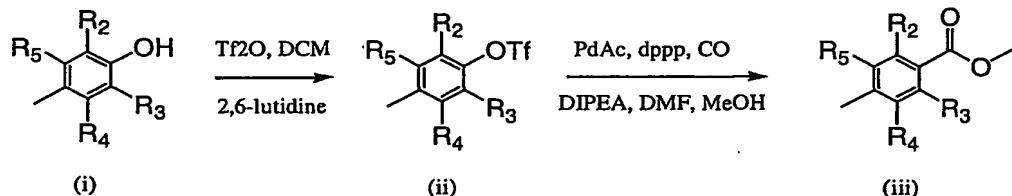


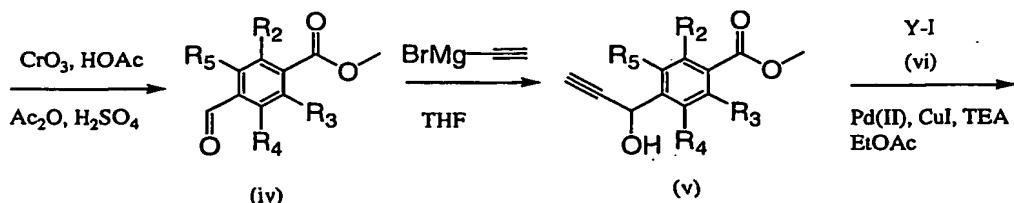
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Referring to scheme 4, starting compound (i), prepared according to the procedures described in scheme 2, is converted to the iodo intermediate (ii) and reacted with alkyne (iii) to give intermediate (iv). Alkyne (iii) is prepared by reacting Y-COOH with Br-C≡CH in THF. Intermediate (iv) is then converted to the alkane (v) by reacting with Rh/Al₂O₃ in H₂ atmosphere and the ester group converted to the free acid by reacting with LiI in pyridine to give (vi). Intermediate (vi) is reacted with amino acid (vii) to give compound of the invention (viii). In a particular embodiment of scheme 4, Y is phenyl optionally substituted with alkyl, hydroxy or halogen. In a particularly preferred embodiment Y is 3-chloro-phenyl or 3-hydroxy-phenyl.

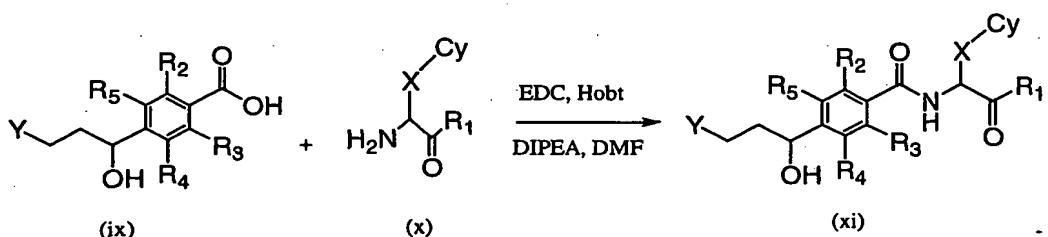
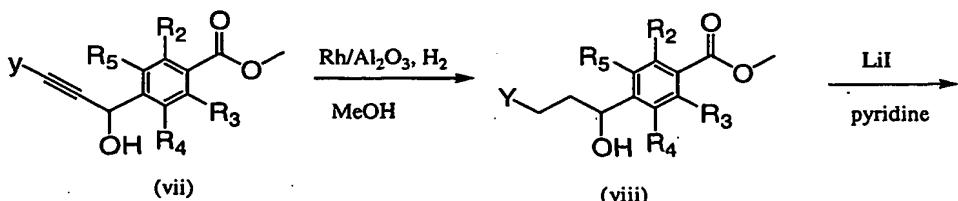
In another particular embodiment, compounds of formula (Ie) of the invention may be prepared according to scheme 5.

Scheme 5





5



Referring to scheme 5, starting compound (i) is reacted with triflic anhydride and 2,6-lutidine to give intermediate (ii) which is converted to methyl ester (iii) by reacting with palladium(II)acetate, 1,3-bi(diphenylphosphino)propane (dppp) and subsequently with diisopropyl ethylamine (DIPEA) in DMF and methanol. The ester (iii) is then reacted with CrO_3 in acetic acid and anhydride to give aldehyde (iv) which is reacted with Grignard reagent ethynyl-magnesium bromide in THF to give alkyne intermediate (v). Iodo reagent (vi) Y-I is reacted with (v) to give intermediate (vii) which is converted to the alkane (viii) by reacting with $\text{Rh}/\text{Al}_2\text{O}_3$ under hydrogen atmosphere. The methyl ester is converted to free acid (ix) with LiI in pyridine which is then coupled to amino acid residue (x) to give compound of the invention (xi). In preferred embodiments of scheme 5, Y is phenyl, optionally substituted with hydroxy, halogen,

alkyl or alkoxy. In more preferred embodiments, Y is 3-hydroxy-phenyl or 3-chloro-phenyl.

Compounds of the invention bind to LFA-1 preferentially over Mac-1. Accordingly, in an aspect of the invention, there is provided a method of inhibiting the binding of LFA-1 to ICAMs (cellular adhesion molecules), the method comprising contacting LFA-1 with a compound of formula (I). The method may be carried out in vivo or ex vivo as a solution based or cell based assay wherein the compound of the invention is introduced to LFA-1 in the presence of a putative or known ligand (such as ICAM-1). The compound of the invention may be labeled, for example isotopically radiolabeled, or labeled with a fluorophore such as fluorescein isothiocyanate (FITC), to facilitate detection of ligand binding or reduction thereof to the protease. Thus compounds of the invention are useful for diagnostic and screening assays.

5

Compounds of the invention are therapeutically and/or prophylactically useful for treating diseases or conditions mediated by LFA-1 activity. Accordingly in an aspect of the invention, there is provided a method of 10 treating a disease or condition mediated by LFA-1 in a mammal, i.e. a human, comprising administering to said mammal an effective amount of a compound of the invention. By "effective amount" is meant an amount of compound which upon administration is capable of reducing 15 the activity of LFA-1; or the amount of compound required to prevent, inhibit or reduce the severity of any symptom associated with an LFA-1 mediated condition or disease upon administration.

5 Compounds of the invention or compositions thereof are useful in treating conditions or diseases including: psoriasis; responses associated with inflammatory bowel disease (such as Crohn's disease and ulcerative colitis), dermatitis, meningitis, encephalitis, uveitis, allergic
10 conditions such as eczema and asthma, conditions involving infiltration of T-cells and chronic inflammatory responses, skin hypersensitivity reactions (including poison ivy and poison oak); atherosclerosis, autoimmune diseases such as rheumatoid arthritis,
15 systemic lupus erythematosus (SLE), diabetes mellitus, multiple sclerosis, Reynaud's syndrome, autoimmune thyroiditis, experimental autoimmune encephalomyelitis, Sjorgen's syndrome, juvenile onset diabetes, and immune responses associated with delayed hypersensitivity
20 mediated by cytokines and T-lymphocytes typically found in tuberculosis, sarcoidosis, polymyositis, granulomatosis and vasculitis; pernicious anemia; diseases involving leukocyte diapedesis; CNS inflammatory disorder, multiple organ injury syndrome secondary to
25 septicaemia or trauma; autoimmune hemolytic anemia; myasthenia gravis; antigen-antibody complex mediated diseases; all types of transplantations, including graft vs. host or host vs. graft disease, HIV and rhinovirus infection, pulmonary fibrosis, alopecia, scleredoma,
30 endometriosis, vitiligo, ischemic reperfusion injury mediated by neutrophils such as acute myocardial infarction, restenosis following PTCA, invasive procedures such as cardiopulmonary bypass surgery, cerebral edema, stroke, traumatic brain injury,
35 hemorrhagic shock, burns, ischemic kidney disease, multi-organ failure, wound healing and scar formation, atherosclerosis.

5 The actual amount of compound administered and the route
of administration will depend upon the particular disease
or condition as well as other factors such as the size,
age, sex and ethnic origin of the individual being
treated and is determined by routine analysis. In
10 general, intravenous doses will be in the range from
about 0.01-1000 mg/kg of patient body weight per day,
preferably 0.1 to 20 mg/kg and more preferably 0.3 to 15
mg/kg. Administration may be once or multiple times per
day for several days, weeks or years or may be a few
15 times per week for several weeks or years. The amount of
compound administered by other routes will be that which
provides a similar amount of compound in plasma compared
to the intravenous amounts described which will take into
consideration the plasma bioavailability of the
20 particular compound administered.

In methods of the invention, the compound may be
administered orally (including buccal, sublingual,
inhalation), nasally, rectally, vaginally, intravenously
25 (including intra-arterially), intradermally,
subcutaneously, intramuscularly and topically. Compounds
will be formulated into compositions suitable for
administration for example with carriers, diluents,
thickeners, adjuvants etc. as are routine in the
30 formulation art. Accordingly, another aspect of the
invention provides pharmaceutical compositions comprising
a compound of formula (I) and a pharmaceutically
acceptable carrier, excipient or adjuvant and may also
include additional active ingredients such as anti-
35 inflammatories e.g. NSAIDs.

Dosage forms include solutions, powders, tablets,
capsules, gel capsules, suppositories, topical ointments

5 and creams and aerosols for inhalation. Formulations for non-parenteral administration may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives. Pharmaceutically acceptable organic or inorganic carrier substances suitable for non-
10 parenteral administration which do not deleteriously react with compounds of the invention can be used. Suitable pharmaceutically acceptable carriers include, but are not limited to, water, salt solutions, alcohol, polyethylene glycols, gelatin, lactose, amylose,
15 magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose, polyvinylpyrrolidone and the like. The formulations can be sterilized and, if desired, mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers,
20 salts for influencing osmotic pressure, buffers, colorings flavorings and/or aromatic substances and the like which do not deleteriously react with compounds of the invention. Aqueous suspensions may contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. Optionally, the suspension may
25 also contain stabilizers.

Compounds of the invention exhibit high oral bioavailability. Accordingly, in a preferred embodiment, compounds of the invention are administered via oral delivery. Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets, troches, tablets or
30 SECs (soft elastic capsules or caplets). Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids, carrier substances or binders may be desirably added to such formulations. Such formulations may be used to
35

5 effect delivering the compounds to the alimentary canal
for exposure to the mucosa thereof. Accordingly, the
formulation can consist of material effective in
protecting the compound from pH extremes of the stomach,
or in releasing the compound over time, to optimize the
10 delivery thereof to a particular mucosal site. Enteric
coatings for acid-resistant tablets, capsules and caplets
are known in the art and typically include acetate
phthalate, propylene glycol and sorbitan monoleate.

15 Various methods for producing formulations for alimentary
delivery are well known in the art. See, generally
Remington's Pharmaceutical Sciences, 18th Ed., Gennaro,
ed., Mack Publishing Co., Easton, PA, 1990. The
formulations of the invention can be converted in a known
20 manner into the customary formulations, such as tablets,
coated tablets, pills, granules, aerosols, syrups,
emulsions, suspensions and solutions, using inert,
non-toxic, pharmaceutically suitable excipients or
solvents. The therapeutically active compound should in
25 each case be present in a concentration of about 0.1% to
about 99% by weight of the total mixture, that is to say
in amounts which are sufficient to achieve the desired
dosage range. The formulations are prepared, for
example, by extending the active compounds with solvents
30 and/or excipients, if appropriate using emulsifying
agents and/or dispersing agents, and, for example, in the
case where water is used as the diluent, organic solvents
can be used as auxiliary solvents if appropriate.

35 Compositions may also be formulated with binding agents
(e.g., pregelatinised maize starch, polyvinylpyrrolidone
or hydroxypropyl methylcellulose); fillers (e.g.,
lactose, microcrystalline cellulose or calcium hydrogen

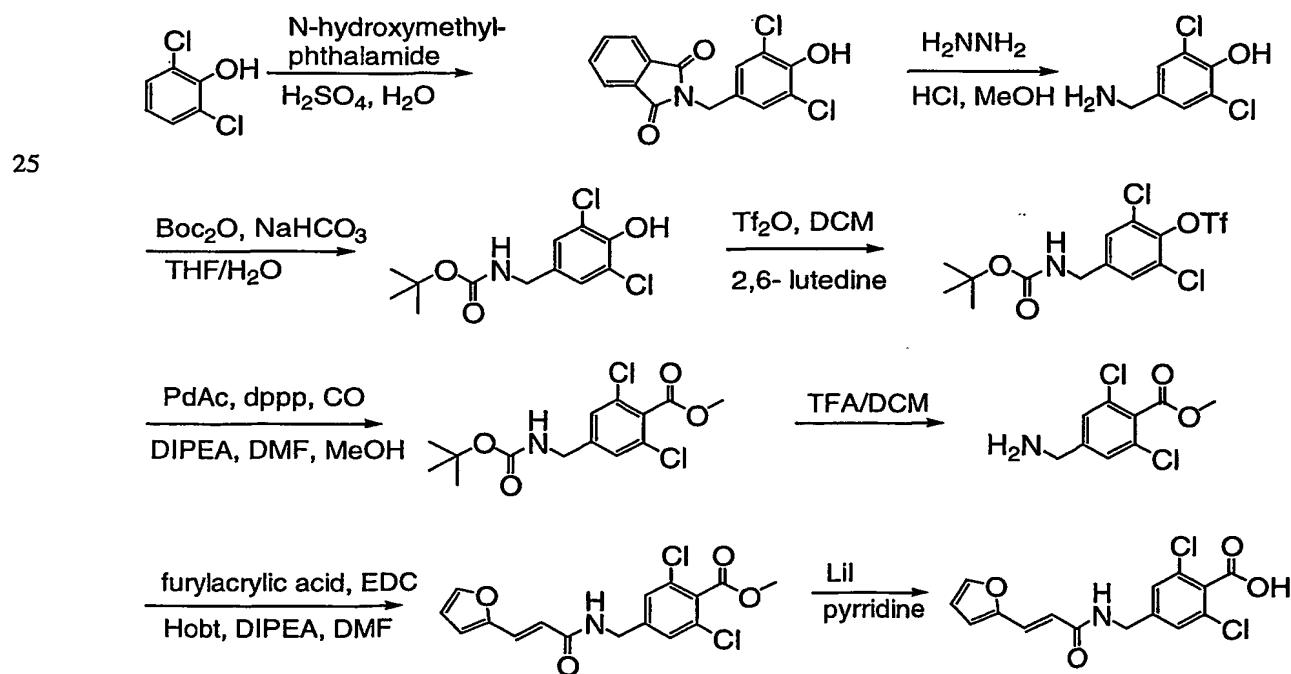
5 phosphate); lubricants (e.g., magnesium stearate, talc or silica); disintegrates (e.g., starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulfate). Tablets may be coated by methods well known in
10 the art. The preparations may also contain flavoring, coloring and/or sweetening agents as appropriate.

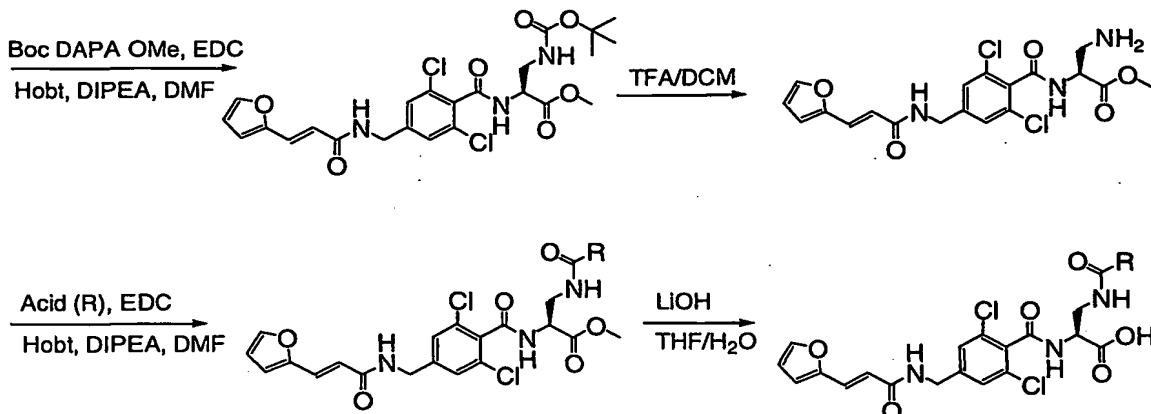
Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing
15 predetermined amounts of the active ingredients; as powders or granules; as solutions or suspensions in an aqueous liquid or a non-aqueous liquid; or as oil-in-water emulsions or water-in-oil liquid emulsions. A tablet may be made by compression or molding,
20 optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine, the active ingredients in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent,
25 preservative, surface active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or
30 controlled release of the active ingredients therein.

5 EXAMPLES

Abbreviations used in the following section: Boc = *t*-butyloxycarbonyl; Boc₂O = *t*-butyloxycarbonyl anhydride; DMA = dimethylacetamide; DMF = dimethylformamide; HObt = 1-hydroxybenztriazole; TFA = trifluoroacetic acid; DCM = dichloromethane; MeOH = methanol; HOAc = acetic acid; HCl = hydrochloric acid; H₂SO₄ = sulfuric acid; K₂CO₃ = potassium carbonate; THF = tetrahydrofuran; EtOAc = ethyl acetate; DIPEA = diisopropylethylamine; NaHCO₃ = sodium bicarbonate; ACN = acetonitrile; Na₂•EDTA = ethylenediaminetetraacetic acid sodium salt; TBAF = tetrabutyl ammonium fluoride; EDC = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide•HCl; TEA = triethylamine; MgSO₄ = magnesium sulfate; TES = triethylsilane; Et₂O = diethyl ether; BBr₃ = boron tribromide

EXAMPLE 1 Synthesis of compounds 16, 17, 38-40, 46-50





A round bottom flask was equipped with an efficient overhead stirrer and charged with concentrated H_2SO_4 (2.7 x volume of H_2O) and H_2O and cooled to $\sim 5^\circ C$ with an ethanol/ice bath. Once cool, 1 equivalent 2.6 dichloro phenol and 1 equivalent of N-(hydroxymethyl)phthalimide were added with vigorous stirring. The reaction was kept cool for 4 hours and then allowed to warm to room temperature overnight with constant stirring. The reaction generally proceeded to a point where there was just a solid in the round bottom flask. At that point EtOAc and H_2O were added and stirred into the solid. Large chunks were broken up and then the precipitate was filtered and washed with more EtOAc and H_2O . The product was then used without further purification after drying overnight under vacuum.

1 equivalent of the dry product and methanol (22.5ml x #g of starting material) was added to a round bottom flask equipped with a H_2O condenser and stirring bar. 1.2 equivalents of hydrazine mono hydrate was added and the mixture refluxed for 4 hours. After cooling to room temperature, concentrated HCl (4.5ml x #g of starting material) was carefully added. Upon completion of the addition, the mixture was refluxed overnight (> 8 hours).

5 The reaction was cooled to 0°C and the precipitated by-product was removed by filtration. The filtrate was then concentrated *in vacuo*.

10 The crude amine residue was dissolved in a 3:2 THF/H₂O solution. 1.1 equivalents of solid NaHCO₃ and 1.1 equivalents of Boc₂O were added and the mixture was stirred overnight. The reaction was concentrated, and the residue was partitioned between H₂O and Et₂O. The aqueous layer was extracted with Et₂O and the combined organic layers were dried over MgSO₄ and concentrated *in vacuo* to a solid. Recrystallization from hot methanol and H₂O provided pure product.

20 1 equivalent of the Boc protected amine and 1.5 equivalents of 2, 6-lutidine was dissolved, with mild heating when necessary, in DCM in a round bottom flask. Once the starting material had completely dissolved, the mixture was cooled to -78°C under N₂ with a dry ice ethanol bath. Once cool, 2.5 equivalents of triflic anhydride was added and the reaction was allowed to slowly come to room temperature with stirring. The reaction was monitored by TLC and was generally done in 4 hours. Upon completion, the reaction was concentrated *in vacuo* and the residue partitioned between EtOAc and H₂O. 30 The organic layer was washed twice with 0.1N H₂SO₄, twice with saturated NaHCO₃, once with brine, dried over MgSO₄ and concentrated *in vacuo*. The residue was then purified on silica gel using DCM as eluent to provide pure triflate.

35

1 equivalent of triflate was dissolved in DMF and MeOH in the glass insert of a high pressure Parr bomb. The starting material was then degassed while stirring with

5 CO for 10 minutes. 0.15 equivalents palladium(II) acetate and 0.15 equivalents of 1, 3- bis(diphenylphosphino) propane were then added and the mixture was then degassed while stirring with CO for another 10 minutes at which time 2.5 equivalents of diisopropyl ethyl amine was
10 added. After properly assembling the bomb, it was charged with 300psi CO gas and heated to 70°C with stirring overnight. The bomb was then cooled and vented. The mixture was transferred to a round bottom flask and concentrated *in vacuo*. The residue was then purified on silica gel using DCM with 1% acetone and 1% TEA as eluent
15 to provide pure methyl ester.

The Boc protected amine was dissolved in a solution of TFA in DCM (1:1). After 20 minutes, the reaction was
20 concentrated *in vacuo*. The resulting oil was dissolved in toluene and then reconcentrated *in vacuo*. The TFA salt of the amine was dissolved in Et₂O and washed twice with a 10% solution of K₂CO₃ in H₂O and once with brine. The organic layer was then dried over MgSO₄, filtered and
25 concentrated *in vacuo*.

1 equivalent of the free based amine, 3 equivalents of furylacrylic acid, 3 equivalents of EDC and 1 equivalent of HObt were dissolved DMA. The reaction was stirred at
30 room temperature and monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was concentrated *in vacuo*. The resulting oil was re suspended in Et₂O and washed twice with 0.1 N H₂SO₄, twice with saturated NaHCO₃, and once with brine. The organic layer was then dried over
35 MgSO₄, filtered and concentrated *in vacuo*. The residue was then purified on silica gel using 5% methanol in DCM as eluent to provide pure methyl ester.

5 2.3 equivalents of lithium iodide was added to 1 equivalent of the methyl ester in pyridine, and the mixture heated at reflux for 8 hours. The reaction was concentrated *in vacuo* and the residue was partitioned between EtOAc and 1N HCl. The aqueous layer was extracted
10 three times with EtOAc, and the combined organic layers were washed with 1M NaHCO₃, dried over MgSO₄ and concentrated *in vacuo*. The residue was dissolved in NMM and the solution concentrated *in vacuo*. The residue was taken up in DCM and then washed three times with 1N HCl.
15 The organic layer was dried over MgSO₄ and concentrated *in vacuo* to provide the benzoic acid in high enough purity to be used without further purification.

20 1 equivalent of the acid, 2 equivalents of commercially available β - Boc- diaminopropionic acid methyl ester, 2 equivalents of EDC, 1 equivalent of Hobt and 3 equivalents of DIPEA were dissolved DMA. The reaction was stirred at room temperature and monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was concentrated
25 *in vacuo*. The resulting oil was re suspended in Et₂O and washed twice with 0.1 N H₂SO₄, twice with saturated NaHCO₃, and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was then purified on silica gel using 5% methanol
30 in DCM as eluent to provide pure methyl ester.

The Boc protected amine was dissolved in a solution of TFA in DCM (1:1). After 20 minutes, the reaction was concentrated *in vacuo*. The resulting oil was dissolved in toluene and then reconcentrated *in vacuo*. 1 equivalent of this amine, 2 equivalents of the appropriate commercially available carboxylic acid (compound 16, N- acetyl-D-proline; compound 17, N- acetyl-L-proline; compound 38,

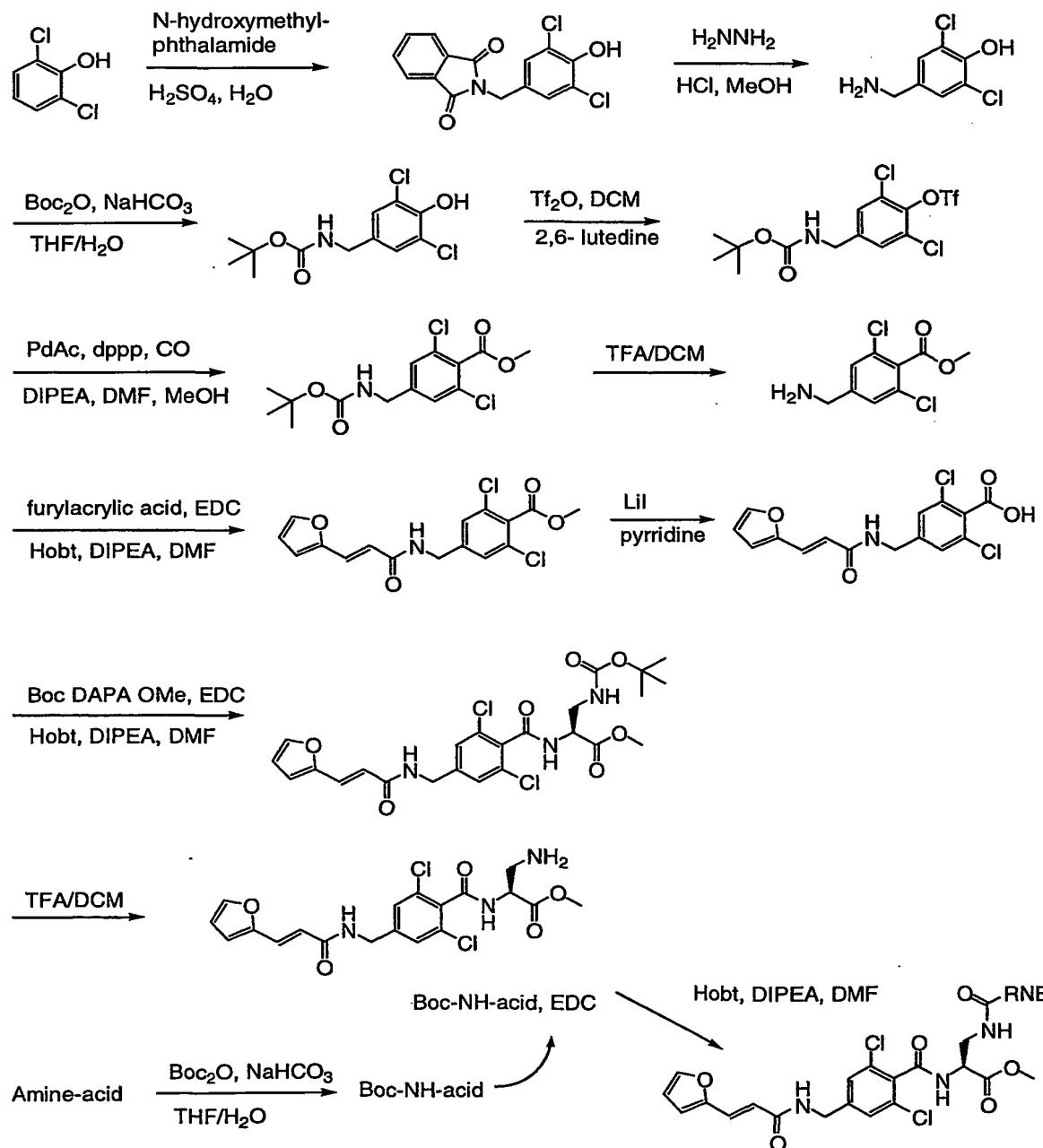
5 (-)-2-oxo-4-thiazolidinecarboxylic acid; compound 39, 1-
cyclohexene-1-carboxylic acid; compound 40, (4R)-(-)-2-
thioxo-4-thiazolidinecarboxylic acid; compound 45,
cyclobutanecarboxylic acid; compound 46, cyclopentane-
carboxylic acid; compound 47, cyclohexanecarboxylic acid;
10 compound 48, 3,4-dihydro-2,2-dimethyl-4-oxo-2H-pyran-6-
carboxylic acid; compound 49, ethyl 1,3-dithiolane-2-
carboxylate (2 equivalents of the ethyl ester was
saponified with 3 equivalents of LiOH•H₂O in THF/H₂O (3/1)
The reaction was monitored by TLC (9/1 DCM/MeOH). Upon
15 completion, the mixture was acidified to pH 2 with 1M HCl
and then concentrated *in vacuo*. The resulting solid was
used without further purification); compound 50,
cyclopropanecarboxylic acid; compound 51, tetrahydro-2-
furoic acid), 2 equivalents of EDC, 1 equivalent of HObt
20 and 3 equivalents of DIPEA were dissolved DMA. The
reaction was stirred at room temperature and monitored by
TLC (9/1 DCM/MeOH). Upon completion, the mixture was
concentrated *in vacuo*. The resulting oil was re suspended
25 in Et₂O and washed twice with 0.1 N H₂SO₄, twice with
saturated NaHCO₃, and once with brine. The organic layer
was then dried over MgSO₄, filtered and concentrated *in
vacuo*. The residue was then purified on silica gel using
5% methanol in DCM as eluent to provide pure methyl
ester.

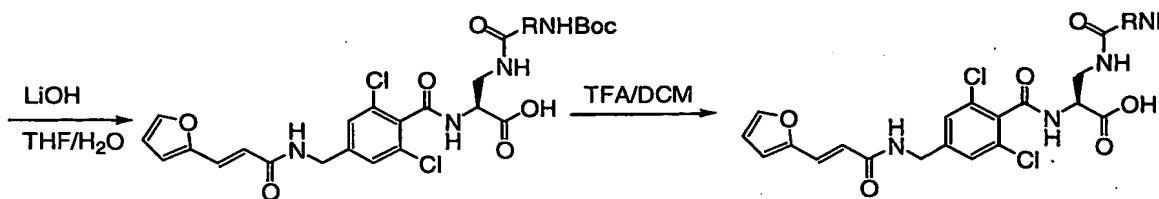
30 1 equivalent of the resultant methyl ester was dissolved
in THF/H₂O (3/1) and 3 equivalents of LiOH•H₂O was added.
The reaction was monitored by TLC (9/1 DCM/MeOH). Upon
completion, the mixture was acidified to pH 2 with 1M HCl
35 and then concentrated *in vacuo*. The resulting solid was
re suspended in Et₂O and washed twice with 0.1 M HCl and
once with brine. The organic layer was then dried over
MgSO₄, filtered and concentrated *in vacuo*. The resulting

5 acid was then purified by reverse phase HPLC, verified by electrospray mass spectrometry and lyophilized to a powder.

10

EXAMPLE 2 Synthesis of compounds 1-15, 41, 43





5

A round bottom flask was equipped with an efficient overhead stirrer and charged with concentrated H_2SO_4 (2.7 x volume of H_2O) and H_2O and cooled to $\sim 5^\circ\text{C}$ with an ethanol/ice bath. Once cool, 1 equivalent 2.6 dichloro phenol and 1 equivalent of N-(hydroxymethyl)phthalimide were added with vigorous stirring. The reaction was kept cool for 4 hours and then allowed to warm to room temperature overnight with constant stirring. The reaction generally proceeds to a point where there was just a solid in the round bottom flask. At this point EtOAc and H_2O were added and stirred into the solid. Large chunks were broken up and then the precipitate was filtered and washed with more EtOAc and H_2O . The product was then used without further purification after drying overnight under vacuum.

1 equivalent of the dry product and methanol (22.5ml x #g of starting material) was added to a round bottom flask equipped with a H_2O condenser and stirring bar. 1.2 equivalents of hydrazine mono hydrate was added and the mixture refluxed for 4 hours. After cooling to room temperature, concentrated HCl (4.5ml x #g of starting material) was carefully added. Upon completion of the addition, the mixture was refluxed overnight (> 8 hours). The reaction was cooled to 0°C and the precipitated by-product was removed by filtration. The filtrate was then concentrated *in vacuo*.

5 The crude amine residue was dissolved in a 3:2 THF/H₂O solution. 1.1 equivalents of solid NaHCO₃ and 1.1 equivalents of Boc₂O were added and the mixture was stirred overnight. The reaction was concentrated, and the residue was partitioned between H₂O and Et₂O. The aqueous
10 layer was extracted with Et₂O and the combined organic layers were dried over MgSO₄ and concentrated *in vacuo* to a solid. Recrystallization from hot methanol and H₂O provided pure product.

15 1 equivalent of the Boc protected amine and 1.5 equivalents of 2, 6- lutidine was dissolved, with mild heating when necessary, in DCM in a round bottom flask. Once the starting material had completely dissolved, the mixture was cooled to -78°C under N₂ with a dry ice
20 ethanol bath. Once cool, 2.5 equivalents of triflic anhydride was added and the reaction was allowed to slowly come to room temperature with stirring. The reaction was monitored by TLC and was generally done in 4 hours. Upon completion, the reaction was concentrated *in vacuo* and the residue partitioned between EtOAc and H₂O.
25 The organic layer was washed twice with 0.1N H₂SO₄, twice with saturated NaHCO₃, once with brine, dried over MgSO₄ and concentrated *in vacuo*. The residue was then purified on silica gel using DCM as eluent to provide pure
30 triflate.

1 equivalent of triflate was dissolved in DMF and MeOH in the glass insert of a high pressure Parr bomb. The starting material was then degassed while stirring with
35 CO for 10 minutes. 0.15 equivalents palladium(II) acetate and 0.15 equivalents of 1, 3- bis(diphenylphosphino) propane were then added and the mixture was then degassed while stirring with CO for another 10 minutes at which

5 time 2.5 equivalents of diisopropyl ethyl amine was added. After properly assembling the bomb, it was charged with 300psi CO gas and heated to 70°C with stirring overnight. The bomb was then cooled and vented. The mixture was transferred to a round bottom flask and
10 concentrated *in vacuo*. The residue was then purified on silica gel using DCM with 1% acetone and 1% TEA as eluent to provide pure methyl ester.

15 The Boc protected amine was dissolved in a solution of TFA in DCM (1:1). After 20 minutes, the reaction was concentrated *in vacuo*. The resulting oil was dissolved in toluene and then reconcentrated *in vacuo*. The TFA salt of the amine was dissolved in Et₂O and washed twice with a 10% solution of K₂CO₃ in H₂O and once with brine. The
20 organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*.

25 1 equivalent of the free based amine, 3 equivalents of furylacrylic acid, 3 equivalents of EDC and 1 equivalent of Hobt were dissolved DMA. The reaction was stirred at room temperature and monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was concentrated *in vacuo*. The resulting oil was re suspended in Et₂O and washed twice with 0.1 N H₂SO₄, twice with saturated NaHCO₃, and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was then purified on silica get using 5% methanol in DCM as eluent to provide pure methyl ester.

35 2.3 equivalents of lithium iodide was added to 1 equivalent of the methyl ester in pyridine, and the mixture heated at reflux for 8 hours. The reaction was concentrated *in vacuo* and the residue was partitioned

5 between EtOAc and 1N HCl. The aqueous layer was extracted
three times with EtOAc, and the combined organic layers
were washed with 1M NaHCO₃, dried over MgSO₄ and
concentrated *in vacuo*. The residue was dissolved in NMM
and the solution concentrated *in vacuo*. The residue was
10 taken up in DCM and then washed three times with 1N HCl.
The organic layer was dried over MgSO₄ and concentrated *in
vacuo* to provide the benzoic acid in high enough purity
to be used without further purification.

15 1 equivalent of the acid, 2 equivalents of commercially
available β - Boc-, diaminopropionic acid methyl ester, 2
equivalents of EDC, 1 equivalent of Hobt and 3
equivalents of DIPEA were dissolved DMA. The reaction was
stirred at room temperature and monitored by TLC (9/1
20 DCM/MeOH). Upon completion, the mixture was concentrated
in vacuo. The resulting oil was re suspended in Et₂O and
washed twice with 0.1 N H₂SO₄, twice with saturated
NaHCO₃, and once with brine. The organic layer was then
dried over MgSO₄, filtered and concentrated *in vacuo*. The
residue was then purified on silica gel using 5% methanol
25 in DCM as eluent to provide pure methyl ester.

The Boc protected amine was dissolved in a solution of
TFA in DCM (1:1). After 20 minutes, the reaction was
30 concentrated *in vacuo*. The resulting oil was dissolved in
toluene and then reconcentrated *in vacuo*. 1 equivalent of
this amine, 2 equivalents of the appropriate commercially
available carboxylic acid ((N-Boc acids were purchased
where available. Other acids were purchased as the free
35 amine and Boc protected by the following procedure: The
amine was dissolved in a 3:2 THF/H₂O solution. 1.1
equivalents of solid NaHCO₃ and 1.1 equivalents of Boc₂O
were added and the mixture was stirred overnight. The

5 reaction was concentrated to remove the THF, and the resulting aqueous layer was partitioned with hexanes. The aqueous layer was then acidified to pH 2 with 1N HCl and then partitioned twice with EtOAc. The combined organic layers were dried over MgSO₄ and concentrated *in vacuo*.
10 The resulting product was used without further purification) compound 1 D,L-pipecolinic acid; compound 2, nipecotic acid; compound 3, isonipecotic acid; compound 4, N-Boc-L-proline; compound 5, N-Boc-D-proline; compound 6, Boc-L-thiazolidine-4-carboxylic acid;
15 compound 7, N-Boc-L-pyroglutamic acid; compound 8, N-Boc-D-pyroglutamic acid; compound 9, L-pipecolinic acid; compound 10, D-cis-4-hydroxyproline; compound 11, L-cis-4-hydroxyproline; compound 12, D-hydroxyproline; compound 13, (2S, 3S)-3-methylpyrrolidine-2-carboxylic acid;
20 compound 14, N-Boc-L-hydroxyproline; compound 15, Boc-D-thiazolidine-4-carboxylic acid; compound 41, L-3-hydroxyproline; compound 43, trans-3-azabicyclo[3.1.0]-hexane-2-carboxylic acid), 2 equivalents of EDC, 1 equivalent of Hobt and 3 equivalents of DIPEA were dissolved DMA. The reaction was stirred at room temperature and monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was concentrated *in vacuo*. The resulting oil was re suspended in Et₂O and washed twice with 0.1 N H₂SO₄, twice with saturated NaHCO₃, and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was then purified on silica gel using 5% methanol in DCM as eluent to provide pure methyl ester.
25
30
35

1 equivalent of the resultant methyl ester was dissolved in THF/H₂O (3/1) and 3 equivalents of LiOH•H₂O was added. The reaction was monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was acidified to pH 2 with 1M HCl

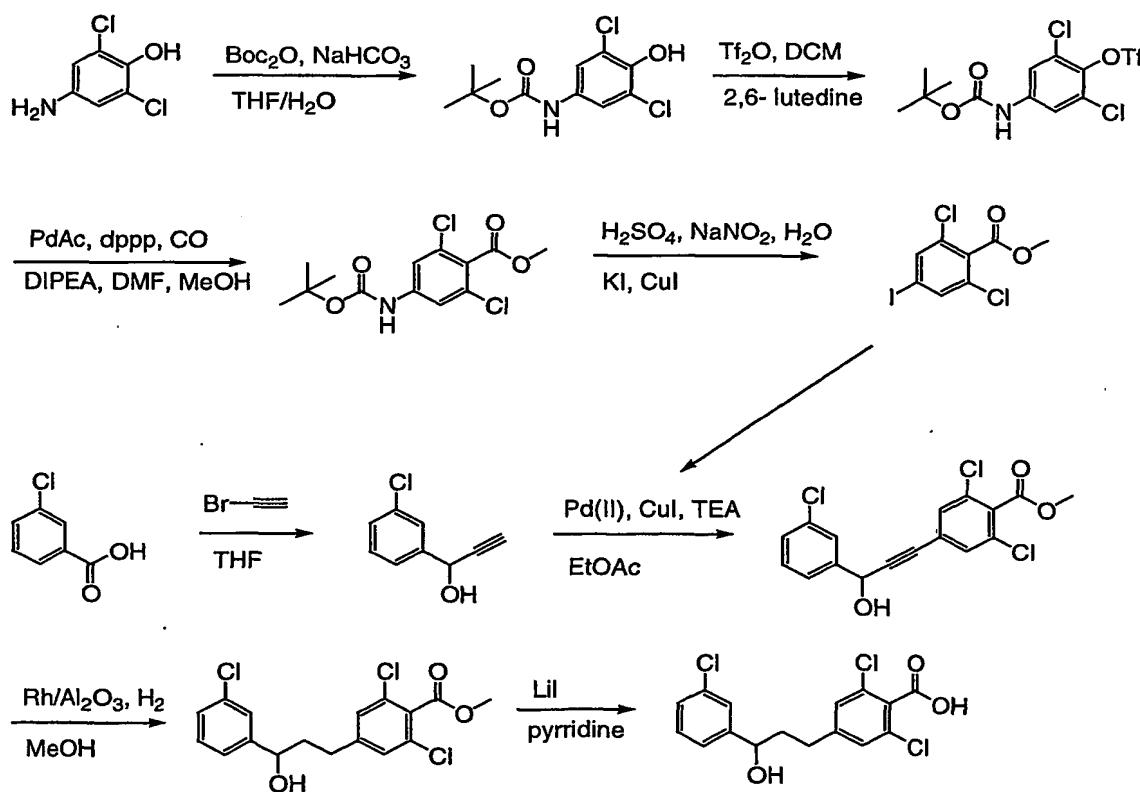
5 and then concentrated *in vacuo*. The resulting solid was re suspended in Et₂O and washed twice with 0.1 M HCl and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*.

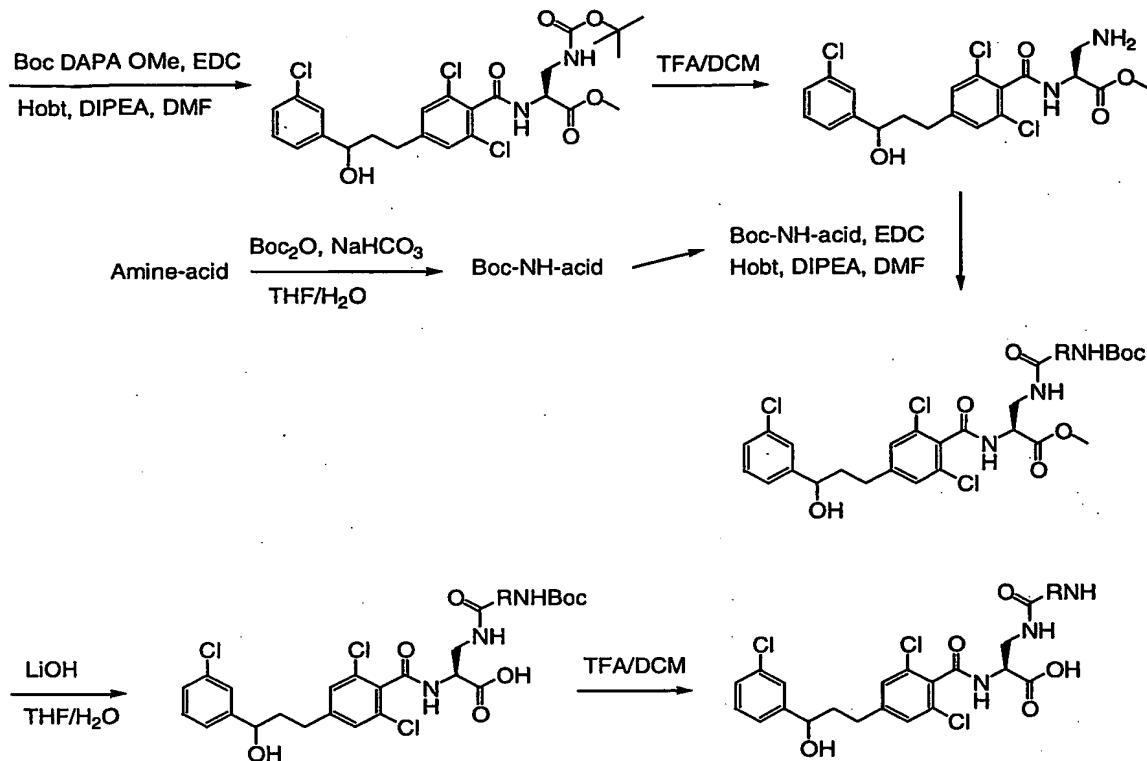
10 Where appropriate the Boc protected residue was dissolved in a solution of TFA in DCM (1:1). After 20 minutes, the reaction was concentrated *in vacuo*. The resulting oil was dissolved in toluene and then reconcentrated *in vacuo*. The resulting acid was then purified by reverse phase HPLC, verified by electrospray mass spectrometry and lyophilized to a powder.

15

EXAMPLE 3 Synthesis of compounds 18-21

20





1 equivalent of 4-amino-2,6-dichlorophenol was dissolved in a 3:2 THF/H₂O solution. 1.1 equivalents of solid NaHCO₃ and 1.1 equivalents of Boc₂O were added and the solution was stirred overnight. The reaction was concentrated, and the residue was partitioned between H₂O and Et₂O. The aqueous layer was extracted with Et₂O and the combined organic layers were dried over MgSO₄ and concentrated *in vacuo* to a solid. Recrystallization out of Et₂O/hexane provided pure product.

1 equivalent of the phenol was dissolved in DCM containing 2.6 equivalents of 2, 6-lutidine and the mixture was cooled to -78°C. After adding 1.25 equivalents of triflic anhydride the stirring reaction was allowed to warm to room temperature overnight. The reaction was then concentrated, and the residue was partitioned between Et₂O and H₂O. The aqueous layer was

5 extracted with Et₂O and the combined organic layers were dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by silica gel flash chromatography (9:1 hexane/Et₂O) to provide the pure triflate.

10 To a stirring solution of 1 equivalent of the triflate in a 2/1 mixture of DMF/MeOH was added 0.15 equivalents of 1, 3-bis(diphenylphosphino)-propane and 2.5 equivalents of TEA. Carbon monoxide gas was bubbled through this solution for 15 minutes, then 0.15 equivalents of Pd(OAc)₂ was added and the reaction was stirred at 70°C for 5-7 hours under an atmosphere of CO (using a balloon filled with CO). The reaction was then concentrated *in vacuo*, and the residue was partitioned between Et₂O and H₂O. The aqueous layer was extracted twice with Et₂O and the combined organic layers were dried over MgSO₄, filtered through a plug of silica gel and concentrated *in vacuo*. The residue was purified by silica gel flash chromatography (9:1:0.02 hexane/DCM/Et₂O) to provide the pure methyl ester.

25 1 equivalent of the Boc-aniline was dissolved in methanol and the solution saturated with HCl. The reaction was heated at 50°C for 3h, then concentrated *in vacuo*. The pale yellow solid was heated in 35% H₂SO₄ until complete dissolution occurred. Upon cooling the mixture by the addition of ice H₂O the amine bisulfate precipitated. The reaction flask was cooled in an ice bath and the mixture stirred vigorously while 1.1 equivalents of sodium nitrite in H₂O was added drop wise. The reaction was stirred at 0°C for another 1.5 hours. An aqueous solution of 10 equivalents of KI was added, followed immediately with 17 equivalents CuI. The reaction was stirred at room temperature for 14 hours, then extracted 3 times with

5 Et₂O. The combined organic layers were washed with 1M NaHCO₃, brine, and dried over MgSO₄, then concentrated *in vacuo*. The residue was purified by silica gel flash chromatography (95:5 hexane/Et₂O) to provide the pure aryl iodide methyl ester.

10 A solution of 1 equivalent of 3-Chlorobenzaldehyde in THF was cooled to -78°C and 1.1 equivalents of 0.5M ethynylmagnesium bromide/THF was added. After stirring the reaction at room temperature for 3 hours, it was 15 diluted with Et₂O and washed twice with 10% citric acid. The combined aqueous layers were back-extracted once with Et₂O. The combined organic layers were washed twice with saturated aqueous NaHCO₃, dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by silica 20 gel flash chromatography (4:1 to 3:2 hexane/Et₂O) to provide the pure alkyne.

25 1 equivalent of the aryl iodide methyl ester was dissolved in EtOAc and the solution was degassed by passing N₂ through a pipette and into the solution for 10 minutes. 1.25 equivalents of the alkyne was added, followed by 0.02 equivalents of dichlorobis(triphenylphosphine)palladium(II), 0.04 equivalents of CuI and 5 equivalents TEA. The reaction 30 was stirred for 14 hours, diluted with EtOAc, washed twice with 5% Na₂•EDTA, brine and then dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by silica gel flash chromatography (gradient elution, using Et₂O to EtOAc) to provide the pure aryl alkyne.

35 1 equivalent of the aryl alkyne was dissolved in MeOH and the solution was degassed by passing N₂ through a pipette and into the solution for 10 minutes. The 5% Rh/Al₂O₃ was

5 added, one balloon-full of hydrogen was passed through
the solution, and the reaction was stirred under an
atmosphere of H₂ (using a balloon) for 7 hours, after
which the reaction was filtered through a pad of celite
10 and concentrated *in vacuo*. The residue was purified by
silica gel flash chromatography (gradient elution, using
Et₂O to EtOAc) to provide the pure product.

2.3 equivalents of lithium iodide was added to 1
equivalent of the methyl ester in pyridine, and the
15 mixture heated at reflux for 8 hours. The reaction was
concentrated *in vacuo* and the residue was partitioned
between EtOAc and 1N HCl. The aqueous layer was extracted
three times with EtOAc, and the combined organic layers
were washed with 1M NaHCO₃, dried over MgSO₄ and
20 concentrated *in vacuo*. The residue was dissolved in NMM
and the solution concentrated *in vacuo*. The residue was
taken up in DCM and then washed three times with 1N HCl.
The organic layer was dried over MgSO₄ and concentrated *in
25 vacuo* to provide the benzoic acid in high enough purity
to be used without further purification.

1 equivalent of the acid, 2 equivalents of commercially
available β - Boc- diaminopropionic acid methyl ester, 2
equivalents of EDC, 1 equivalent of Hobt and 3
30 equivalents of DIPEA were dissolved DMA. The reaction was
stirred at room temperature and monitored by TLC (9/1
DCM/MeOH). Upon completion, the mixture was concentrated
in vacuo. The resulting oil was re suspended in Et₂O and
washed twice with 0.1 N H₂SO₄, twice with saturated
35 NaHCO₃, and once with brine. The organic layer was then
dried over MgSO₄, filtered and concentrated *in vacuo*. The
residue was then purified on silica gel using 5% methanol
in DCM as eluent to provide pure methyl ester.

5

The Boc protected amine was dissolved in a solution of TFA in DCM (1:1). After 20 minutes, the reaction was concentrated *in vacuo*. The resulting oil was dissolved in toluene and then reconcentrated *in vacuo*. 1 equivalent of
10 this amine, 2 equivalents of the appropriate commercially available carboxylic acid ((N-Boc acids were purchased where available. Other acids were purchased as the free amine and Boc protected by the following procedure: The amine was dissolved in a 3:2 THF/H₂O solution. 1.1
15 equivalents of solid NaHCO₃ and 1.1 equivalents of Boc₂O were added and the mixture was stirred overnight. The reaction was concentrated to remove the THF, and the resulting aqueous layer was partitioned with hexanes. The aqueous layer was then acidified to pH 2 with 1N HCl and then partitioned twice with EtOAc. The combined organic layers were dried over MgSO₄ and concentrated *in vacuo*.
20 The resulting product was used without further purification) example 18, N-Boc-D-proline; example 19, N-Boc-L-proline; example 20, Boc-L-thiazolidine-4-carboxylic acid; example 21, isonipecotic acid; 2
25 equivalents of EDC, 1 equivalent of Hobt and 3 equivalents of DIPEA were dissolved DMA. The reaction was stirred at room temperature and monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was concentrated
30 *in vacuo*. The resulting oil was re suspended in Et₂O and washed twice with 0.1 N H₂SO₄, twice with saturated NaHCO₃, and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was then purified on silica gel using 5% methanol
35 in DCM as eluent to provide pure methyl ester.

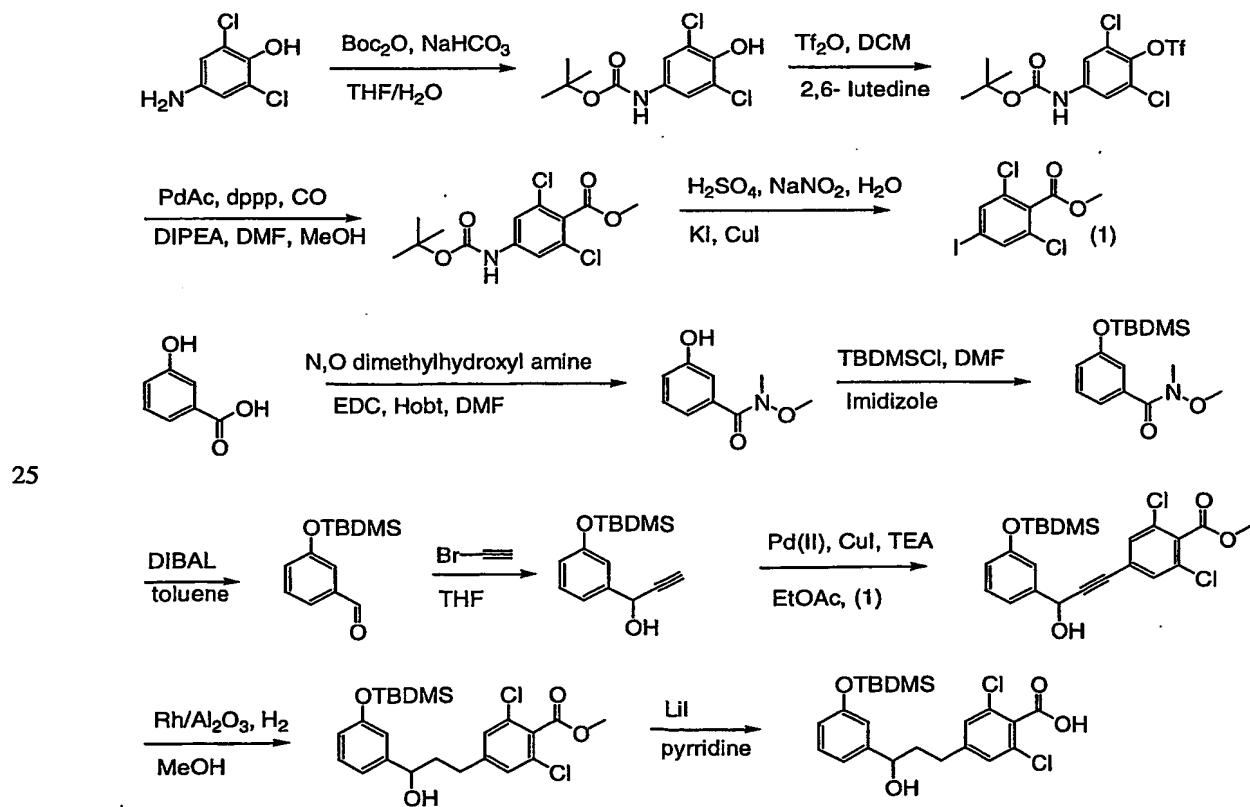
1 equivalent of the resultant methyl ester was dissolved in THF/H₂O (3/1) and 3 equivalents of LiOH•H₂O was added.

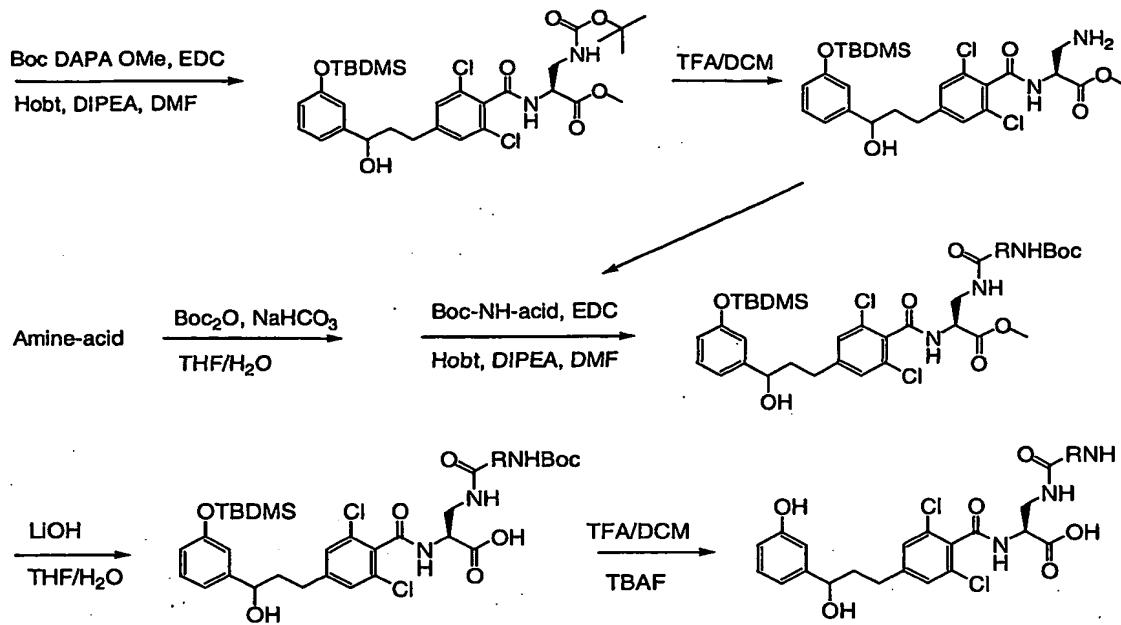
5 The reaction was monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was acidified to pH 2 with 1M HCl and then concentrated *in vacuo*. The resulting solid was re suspended in Et₂O and washed twice with 0.1 M HCl and once with brine. The organic layer was then dried over 10 MgSO₄, filtered and concentrated *in vacuo*.

The Boc protected residue was dissolved in a solution of TFA in DCM (1:1). After 20 minutes, the reaction was concentrated *in vacuo*. The resulting oil was dissolved in toluene and then reconcentrated *in vacuo*. The resulting acid was then purified by reverse phase HPLC, verified by electrospray mass spectrometry and lyophilized to a powder.

20

EXAMPLE 4 Synthesis of compounds 22-25





1 equivalent of 4-amino-2, 6-dichlorophenol was dissolved in a 3:2 THF/H₂O solution. 1.1 equivalents of solid NaHCO₃ and 1.1 equivalents of Boc₂O were added and the solution was stirred overnight. The reaction was concentrated, and the residue was partitioned between H₂O and Et₂O. The aqueous layer was extracted with Et₂O and the combined organic layers were dried over MgSO₄ and concentrated *in vacuo* to a solid. Recrystallization out of Et₂O/hexane provided pure product.

1 equivalent of the phenol was dissolved in DCM containing 2.6 equivalents of 2, 6-lutidine and the mixture was cooled to -78°C. After adding 1.25 equivalents of triflic anhydride the stirring reaction was allowed to warm to room temperature overnight. The reaction was then concentrated, and the residue was partitioned between Et₂O and H₂O. The aqueous layer was extracted with Et₂O and the combined organic layers were dried over MgSO₄ and concentrated *in vacuo*. The residue

5 was purified by silica gel flash chromatography (9:1 hexane/Et₂O) to provide the pure triflate.

To a stirring solution of 1 equivalent of the triflate in a 2/1 mixture of DMF/MeOH was added 0.15 equivalents of 10 1, 3-bis(diphenylphosphino)-propane and 2.5 equivalents of TEA. Carbon monoxide gas was bubbled through this solution for 15 minutes, then 0.15 equivalents of Pd(OAc)₂ was added and the reaction was stirred at 70°C for 5-7 hours under an atmosphere of CO (using a balloon 15 filled with CO). The reaction was then concentrated *in vacuo*, and the residue was partitioned between Et₂O and H₂O. The aqueous layer was extracted twice with Et₂O and the combined organic layers were dried over MgSO₄, 20 filtered through a plug of silica gel and concentrated *in vacuo*. The residue was purified by silica gel flash chromatography (9:1:0.02 hexane/DCM/Et₂O) to provide the pure methyl ester.

25 1 equivalent of the Boc-aniline was dissolved in methanol and the solution saturated with HCl. The reaction was heated at 50°C for 3h, then concentrated *in vacuo*. The pale yellow solid was heated in 35% H₂SO₄ until complete dissolution occurred. Upon cooling the mixture by the addition of ice H₂O the amine bisulfate precipitated. The 30 reaction flask was cooled in an ice bath and the mixture stirred vigorously while 1.1 equivalents of sodium nitrite in H₂O was added drop wise. The reaction was stirred at 0°C for another 1.5 hours. An aqueous solution of 10 equivalents of KI was added, followed immediately 35 with 17 equivalents CuI. The reaction was stirred at room temperature for 14 hours, then extracted 3 times with Et₂O. The combined organic layers were washed with 1M NaHCO₃, brine, and dried over MgSO₄, then concentrated *in*

5 vacuo. The residue was purified by silica gel flash chromatography (95:5 hexane/Et₂O) to provide the pure aryl iodide methyl ester.

10 1.3 equivalents of DIPEA was added to a heterogeneous mixture of 1 equivalent of 3-hydroxybenzoic acid, 1.3 equivalents of N, O-dimethylhydroxylamine hydrochloride, 1.3 equivalents of HOBt and 1.3 equivalents of EDC stirring in DMF. All solids eventually dissolved as the mixture was stirred at room temperature for 28 hours.
15 After concentrating the mixture, the residue was partitioned between Et₂O and H₂O. The aqueous layer was extracted three times with Et₂O and the combined organic layers were dried over MgSO₄, and concentrated *in vacuo*. The residue was purified by silica gel flash chromatography (Et₂O) to provide the pure hydroxamate.
20

25 1 equivalent of the hydroxamate, 2.2 equivalents of t-butyldimethyl silyl chloride and 3 equivalents of imidazole were dissolved in DMF and stirred at room temperature. The reaction was monitored by TLC (9/1 DCM/MeOH). Upon reaction completion, the mixture was concentrated *in vacuo*. The resulting oil was re suspended in Et₂O and washed twice with saturated NaHCO₃, and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*. The product was then 30 used with out further purification.

35 To a stirred -78°C solution of 1 equivalent of the protected hydroxamate in THF was added a solution of 1.2 equivalents of 1.5 M DIBAL in toluene drop wise. The reaction mixture was stirred for an additional 3 hours at -78°C or until TLC showed clean formation of product, with only a trace of starting material. The reaction was

5 quenched by adding to a separatory funnel containing Et₂O and 0.35M NaHSO₄. The layers were separated. The aqueous layer was extracted three times with ethyl ether. The combined organic layers were washed twice with 1N HCl, saturated aqueous NaHCO₃, and over MgSO₄, filtered through
10 a plug of silica gel, and concentrated *in vacuo*. No further purification of the aldehyde was necessary.

A solution of 1 equivalent of the protected aldehyde in THF was cooled to -78°C and 1.1 equivalents of 0.5M ethynylmagnesium bromide/THF was added. After stirring the reaction at room temperature for 3 hours, it was diluted with Et₂O and washed twice with 10% citric acid. The combined aqueous layers were back-extracted once with Et₂O. The combined organic layers were washed twice with saturated aqueous NaHCO₃, dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by silica gel flash chromatography (4:1 to 3:2 hexane/Et₂O) to provide the pure alkyne.

25 1 equivalent of the aryl iodide methyl ester was dissolved in EtOAc and the solution was degassed by passing N₂ through a pipette and into the solution for 10 minutes. 1.25 equivalents of the alkyne was added, followed by 0.02 equivalents of dichlorobis(triphenylphosphine)palladium(II), 0.04 equivalents of CuI and 5 equivalents TEA. The reaction was stirred for 14 hours, diluted with EtOAc, washed twice with 5% Na₂•EDTA, brine and then dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by silica gel flash chromatography (gradient elution, using Et₂O to EtOAc) to provide the pure aryl alkyne.

5 1 equivalent of the aryl alkyne was dissolved in MeOH and the solution was degassed by passing N₂ through a pipette and into the solution for 10 minutes. The 5% Rh/Al₂O₃ was added, one balloon-full of hydrogen was passed through the solution, and the reaction was stirred under an atmosphere of H₂ (using a balloon) for 7 hours, after which the reaction was filtered through a pad of celite, and concentrated *in vacuo*. The residue was purified by silica gel flash chromatography (gradient elution, using Et₂O to EtOAc) to provide the pure product.

15 2.3 equivalents of lithium iodide was added to 1 equivalent of the methyl ester in pyridine, and the mixture heated at reflux for 8 hours. The reaction was concentrated *in vacuo* and the residue was partitioned between EtOAc and 1N HCl. The aqueous layer was extracted three times with EtOAc, and the combined organic layers were washed with 1M NaHCO₃, dried over MgSO₄ and concentrated *in vacuo*. The residue was dissolved in NMM and the solution concentrated *in vacuo*. The residue was taken up in DCM and then washed three times with 1N HCl. The organic layer was dried over MgSO₄ and concentrated *in vacuo* to provide the benzoic acid in high enough purity to be used without further purification.

30 1 equivalent of the acid, 2 equivalents of commercially available β - Boc- diaminopropionic acid methyl ester, 2 equivalents of EDC, 1 equivalent of Hobt and 3 equivalents of DIPEA were dissolved DMA. The reaction was stirred at room temperature and monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was concentrated *in vacuo*. The resulting oil was re suspended in Et₂O and washed twice with 0.1 N H₂SO₄, twice with saturated NaHCO₃, and once with brine. The organic layer was then

5 dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was then purified on silica gel using 5% methanol in DCM as eluent to provide pure methyl ester.

The Boc protected amine was dissolved in a solution of TFA in DCM (1:1). After 20 minutes, the reaction was concentrated *in vacuo*. The resulting oil was dissolved in toluene and then reconcentrated *in vacuo*. 1 equivalent of this amine, 2 equivalents of the appropriate commercially available carboxylic acid ((N-Boc acids were purchased where available. Other acids were purchased as the free amine and Boc protected by the following procedure: The amine was dissolved in a 3:2 THF/H₂O solution. 1.1 equivalents of solid NaHCO₃ and 1.1 equivalents of Boc₂O were added and the mixture was stirred overnight. The reaction was concentrated to remove the THF, and the resulting aqueous layer was partitioned with hexanes. The aqueous layer was then acidified to pH 2 with 1N HCl and then partitioned twice with EtOAc. The combined organic layers were dried over MgSO₄ and concentrated *in vacuo*. The resulting product was used without further purification) example 22, N-Boc-L-proline; example 23, N-Boc-D-proline; example 24, Boc-L-thiazolidine-4-carboxylic acid; example 25, D-hydroxy proline; 2 equivalents of EDC, 1 equivalent of HOBt and 3 equivalents of DIPEA were dissolved DMA. The reaction was stirred at room temperature and monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was concentrated *in vacuo*. The resulting oil was re suspended in Et₂O and washed twice with 0.1 N H₂SO₄, twice with saturated NaHCO₃, and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was then purified on silica gel using 5% methanol in DCM as eluent to provide pure methyl ester.

5

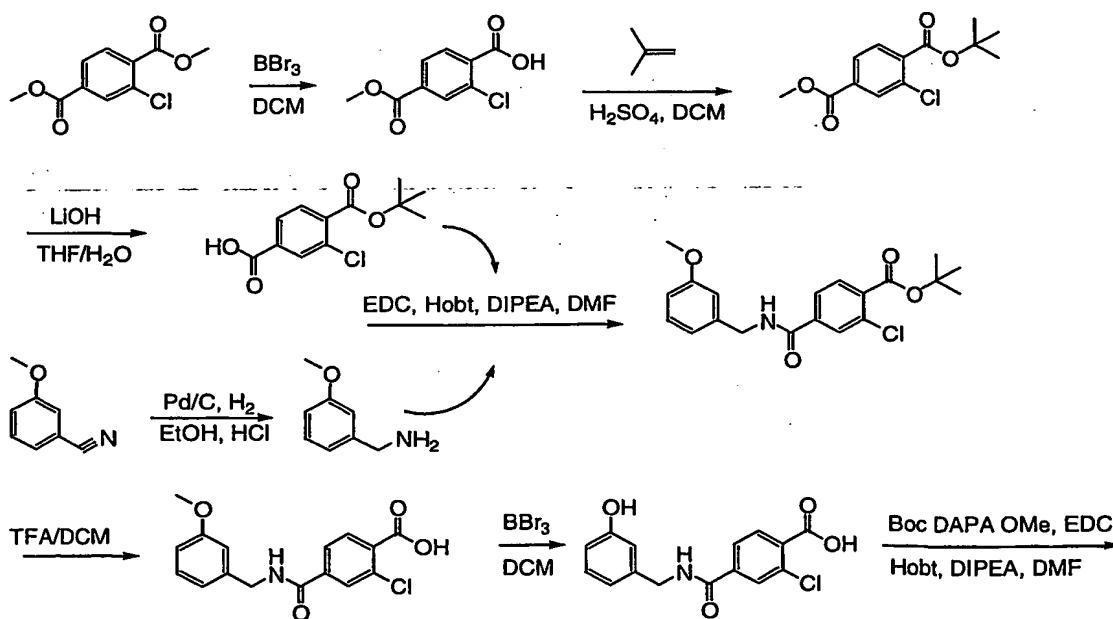
1 equivalent of the resultant methyl ester was dissolved in THF/H₂O (3/1) and 3 equivalents of LiOH·H₂O was added. The reaction was monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was acidified to pH 2 with 1M HCl and then concentrated *in vacuo*. The resulting solid was re suspended in Et₂O and washed twice with 0.1 M HCl and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*.

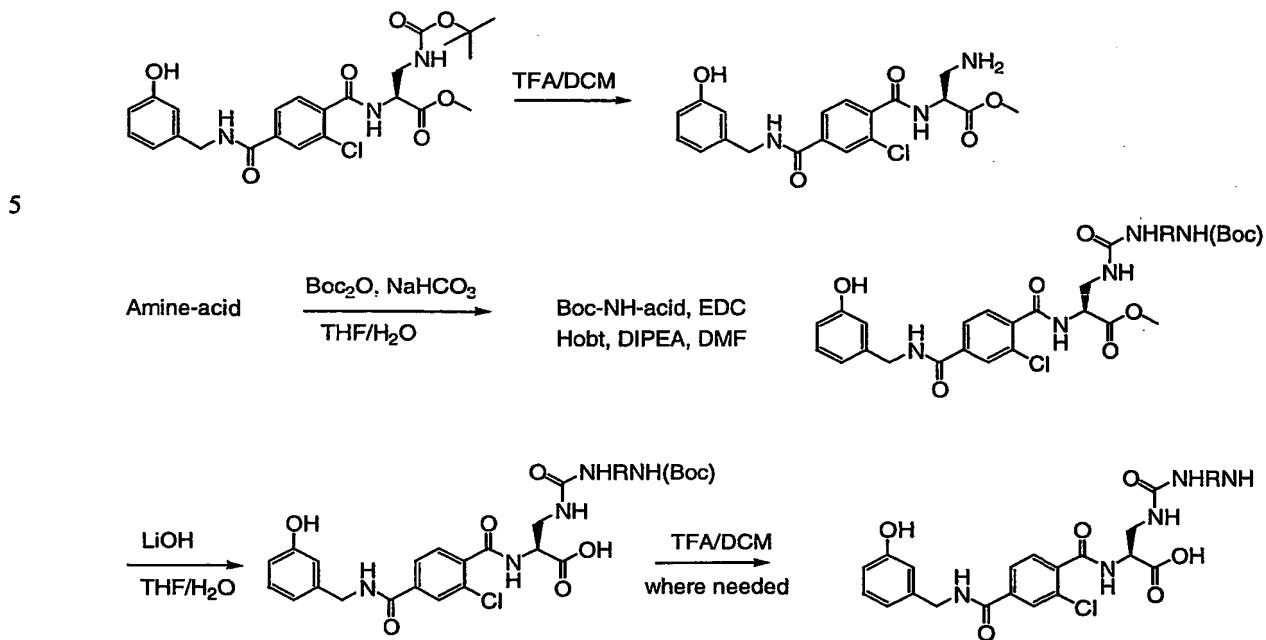
The Boc, silyl residue was dissolved in a solution of TFA in DCM (1:1) with 3 equivalents of TBAF. After 20 minutes, the reaction was concentrated *in vacuo*. The resulting oil was dissolved in toluene and then reconcentrated *in vacuo*. The resulting acid was then purified by reverse phase HPLC, verified by electrospray mass spectrometry and lyophilized to a powder.

EXAMPLE 5

Synthesis of compounds 26-28, 31

25





1 equivalent of dimethyl 2- chloroterephthalic acid was dissolved in DCM and cooled to -5°C in an ice/acetone bath under nitrogen. 1 equivalent of BBr₃ was added drop wise as a solution in DCM over 30 minutes. The reaction was warmed to room temperature and stirred until complete by TLC (DCM/2% HOAc/2% MeOH). The solution was poured onto ice, and the ice was allowed to melt. The mixture was then partitioned with EtOAc and concentrated *in vacuo*. This product was dissolved in H₂O with the addition of saturated NaHCO₃ until the pH remained above 8. This solution was partitioned one time with an equal volume of DCM to remove unreacted diester. The basic solution was acidified at 0°C. with concentrated HCl to pH = 1-1.5, and precipitate was extracted twice with equal volumes of EtOAc. The organics were partitioned once with brine and dried over MgSO₄, filtered and concentrated *in vacuo*. Product was 7:1 of the correct regioisomer by HPLC.

5 The monoester was dissolved in DCM and transferred to a
pre-weighed Parr flask containing a stirring bar. The
flask was cooled to -5°C with a dry ice/alcohol bath
under nitrogen. Once cool, ~30 equivalents of isobutylene
was pumped into solution with stirring. 2.1 equivalents
10 of concentrated sulfuric acid was added and the flask was
sealed with a wired rubber stopper and allowed to warm to
room temperature with stirring. The solution was stirred
until clarification (1-2 days). Once the solution was
clear, it was cooled to 0°C in an ice bath. The stopper
15 was removed and the excess isobutylene was blown off with
nitrogen bubbling. Saturated NaHCO₃ was added to
neutralize the acid and the mixture was concentrated *in*
vacuo until no DCM remained. The solution was then
partitioned into EtOAc. The organics were partitioned
20 twice with dilute HCl, twice with saturated NaHCO₃, once
with brine, dried over MgSO₄, filtered and concentrated *in*
vacuo. The resulting product was used with no further
purification.

25 1 equivalent of the methyl ester was dissolved in THF/H₂O
(3/1) and 3 equivalents of LiOH·H₂O was added. The
reaction was monitored by TLC (9/1 DCM/MeOH). Upon
completion, the mixture was acidified carefully to pH 2
with concentrated HCl and then concentrated *in* vacuo to
30 remove the THF. The resulting aqueous layer was washed
twice with Et₂O and the combined organic layers were
washed once with brine. The organic layer was then dried
over MgSO₄, filtered and concentrated *in* vacuo. The
benzoic acid t-butyl ester was used without further
35 purification.

1 equivalent of 3-methoxybenzonitrile was placed in a
Parr bottle with EtOH, 0.02 equivalents of HCl and 10%

5 (w/w) of 10% Pd on carbon. The vessel was placed in the Parr shaker, charged with 50psi H₂, and shaken for 12 hours. The reaction filtered through a pad of celite and diluted 1:10 with Et₂O. Upon standing over night, fine white needles form. The product was filtered, washed with
10 Et₂O and dried *in vacuo*. The resulting amine hydrochloride salt was then used with out further purification.

15 3 equivalents of the benzoic acid t-butyl ester was coupled to 1 equivalent of the amine hydrochloride salt using 3 equivalents EDC, 1 equivalent of Hobt and 3 equivalents of DIPEA in DMA. The reaction was monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was concentrated *in vacuo*. The resulting oil was re suspended in Et₂O and washed twice with 0.1 N H₂SO₄, twice with
20 saturated NaHCO₃, and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*. The product was then purified on silica get using 5% methanol in DCM as eluent to provide pure t-butyl ester.

25 The t-butyl ester was dissolved in a solution of TFA in DCM (1:1). After 20 minutes, the reaction was concentrated *in vacuo*. The resulting oil was dissolved in toluene and then concentrated *in vacuo* twice.

30 The resulting compound was dissolved in DCM and cooled to -5°C in an ice/acetone bath under nitrogen. 2 equivalents of BBr₃ were added drop wise as a solution in DCM over 30 minutes. The reaction was warmed to room temperature and
35 stirred until complete by TLC (DCM/2% HOAc/2% MeOH). The solution was poured onto ice, and the ice was allowed to melt. The mixture was then partitioned twice with EtOAc and the combined organic layers were dried over MgSO₄. The

5 filtrate was then passed over a plug of silica gel and
concentrated *in vacuo* to afford pure benzoic acid.

10 1 equivalent of the benzoic acid, 2 equivalents of
commercially available β - Boc- diaminopropionic acid
methyl ester, 2 equivalents of EDC, 1 equivalent of Hobt
and 3 equivalents of DIPEA were dissolved DMA. The
reaction was stirred at room temperature and monitored by
TLC (9/1 DCM/MeOH). Upon completion, the mixture was
concentrated *in vacuo*. The resulting oil was re suspended
15 in Et₂O and washed twice with 0.1 N H₂SO₄, twice with
saturated NaHCO₃, and once with brine. The organic layer
was then dried over MgSO₄, filtered and concentrated *in
vacuo*. The residue was then purified on silica gel using
20 5% methanol in DCM as eluent to provide pure methyl
ester.

25 The Boc protected amine was dissolved in a solution of
TFA in DCM (1:1). After 20 minutes, the reaction was
concentrated *in vacuo*. The resulting oil was dissolved in
toluene and then re concentrated *in vacuo*. 1 equivalent
of this amine, 2 equivalents of the appropriate
commercially available carboxylic acid ((N-Boc acids were
purchased where available. Other acids were purchased as
the free amine and Boc protected by the following
30 procedure: The amine was dissolved in a 3:2 THF/H₂O
solution. 1.1 equivalents of solid NaHCO₃ and 1.1
equivalents of Boc₂O were added and the mixture was
stirred overnight. The reaction was concentrated to
remove the THF, and the resulting aqueous layer was
35 partitioned with hexanes. The aqueous layer was then
acidified to pH 2 with 1N HCl and then partitioned twice
with EtOAc. The combined organic layers were dried over
MgSO₄ and concentrated *in vacuo*. The resulting product was

5 used without further purification) example 26, cyclohexanecarboxylic acid; example 27, isonipecotic acid; example 28, D,L-pipecolinic acid; example 31, nipecotic acid; 2 equivalents of EDC, 1 equivalent of HObt and 3 equivalents of DIPEA were dissolved DMA. The
10 reaction was stirred at room temperature and monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was concentrated *in vacuo*. The resulting oil was re suspended in Et₂O and washed twice with 0.1 N H₂SO₄, twice with saturated NaHCO₃, and once with brine. The organic layer
15 was then dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was then purified on silica gel using 5% methanol in DCM as eluent to provide pure methyl ester.

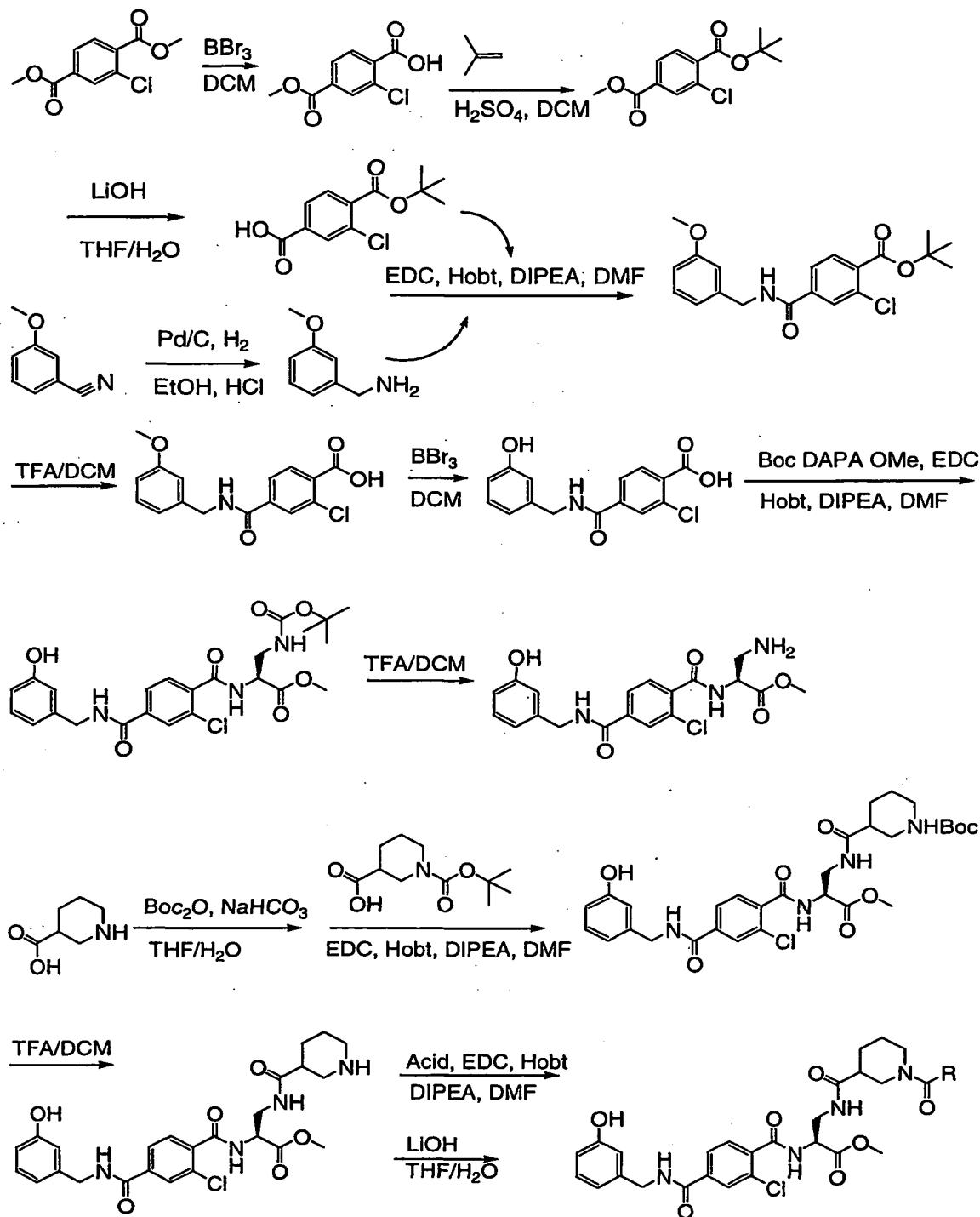
20 1 equivalent of the resultant methyl ester was dissolved in THF/H₂O (3/1) and 3 equivalents of LiOH·H₂O was added. The reaction was monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was acidified to pH 2 with 1M HCl and then concentrated *in vacuo*. The resulting solid was
25 re suspended in Et₂O and washed twice with 0.1 M HCl and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*.

30 Where appropriate the Boc protected residue was dissolved in a solution of TFA in DCM (1:1). After 20 minutes, the reaction was concentrated *in vacuo*. The resulting oil was dissolved in toluene and then re concentrated *in vacuo*. The resulting acid was then purified by reverse phase HPLC, verified by electrospray mass spectrometry and
35 lyophilized to a powder.

5

EXAMPLE 6

Synthesis of compounds 29, 30



1 equivalent of dimethyl 2- chloroterephthalic acid was dissolved in DCM and cooled to -5°C in an ice/acetone bath under nitrogen. 1 equivalent of BBr₃ was added drop

5 wise as a solution in DCM over 30 minutes. The reaction
was warmed to room temperature and stirred until complete
by TLC (DCM/2% HOAc/2% MeOH). The solution was poured
onto ice, and the ice was allowed to melt. The mixture
was then partitioned with EtOAc and concentrated *in*
10 *vacuo*. This product was dissolved in H₂O with the addition
of saturated NaHCO₃ until the pH remained above 8. This
solution was partitioned one time with an equal volume
of DCM to remove unreacted diester. The basic solution
was acidified at 0°C. with concentrated HCl to pH = 1-
15 1.5, and precipitate was extracted twice with equal
volumes of EtOAc. The organics were partitioned once
with brine and dried over MgSO₄, filtered and concentrated
in vacuo. Product was 7:1 of the correct regioisomer by
HPLC.

20 The monoester was dissolved in DCM and transferred to a
pre-weighed Parr flask containing a stirring bar. The
flask was cooled to -5°C with a dry ice/alcohol bath
under nitrogen. Once cool, ~30 equivalents of isobutylene
25 was pumped into solution with stirring. 2.1 equivalents
of concentrated sulfuric acid was added and the flask was
sealed with a wired rubber stopper and allowed to warm to
room temperature with stirring. The solution was stirred
until clarification (1-2 days). Once the solution was
30 clear, it was cooled to 0°C in an ice bath. The stopper
was removed and the excess isobutylene was blown off with
nitrogen bubbling. Saturated NaHCO₃ was added to
neutralize the acid and the mixture was concentrated *in*
vacuo until no DCM remained. The solution was then
35 partitioned into EtOAc. The organics were partitioned
twice with dilute HCl, twice with saturated NaHCO₃, once
with brine, dried over MgSO₄, filtered and concentrated *in*

5 vacuo. The resulting product was used with no further
purification.

10 1 equivalent of the methyl ester was dissolved in THF/H₂O
(3/1) and 3 equivalents of LiOH·H₂O were added. The
reaction was monitored by TLC (9/1 DCM/MeOH). Upon
completion, the mixture was acidified carefully to pH 2
with concentrated HCl and then concentrated *in vacuo* to
remove the THF. The resulting aqueous layer was washed
twice with Et₂O and the combined organic layers were
15 washed once with brine. The organic layer was then dried
over MgSO₄, filtered and concentrated *in vacuo*. The
benzoic acid t-butyl ester was used without further
purification.

20 1 equivalent of 3-methoxybenzonitrile was placed in a
Parr bottle with EtOH, 0.02 equivalents of HCl and 10%
(w/w) of 10% Pd on carbon. The vessel was placed in the
Parr shaker, charged with 50psi H₂, and shaken for 12
hours. The reaction filtered through a pad of celite and
25 diluted 1:10 with Et₂O. Upon standing over night, fine
white needles form. The product was filtered, washed with
Et₂O and dried *in vacuo*. The resulting amine hydrochloride
salt was then used with out further purification.

30 3 equivalents of the benzoic acid t-butyl ester was
coupled to 1 equivalent of the amine hydrochloride salt
using 3 equivalents EDC, 1 equivalent of HObt and 3
equivalents of DIPEA in DMA. The reaction was monitored
by TLC (9/1 DCM/MeOH). Upon completion, the mixture was
35 concentrated *in vacuo*. The resulting oil was re suspended
in Et₂O and washed twice with 0.1 N H₂SO₄, twice with
saturated NaHCO₃, and once with brine. The organic layer
was then dried over MgSO₄, filtered and concentrated in

5 vacuo. The product was then purified on silica gel using
5% methanol in DCM as eluent to provide pure t-butyl
ester.

10 The t-butyl ester was dissolved in a solution of TFA in
DCM (1:1). After 20 minutes, the reaction was
concentrated *in vacuo*. The resulting oil was dissolved in
toluene and then concentrated *in vacuo* twice.

15 The resulting compound was dissolved in DCM and cooled to
-5°C in an ice/acetone bath under nitrogen. 2 equivalents
of BBr₃ were added drop wise as a solution in DCM over 30
minutes. The reaction was warmed to room temperature and
stirred until complete by TLC (DCM/2% HOAc/2% MeOH). The
solution was poured onto ice, and the ice was allowed to
20 melt. The mixture was then partitioned twice with EtOAc
and the combined organic layers were dried over MgSO₄. The
filtrate was then passed over a plug of silica gel and
concentrated *in vacuo* to afford pure benzoic acid.

25 1 equivalent of the benzoic acid, 2 equivalents of
commercially available D- Boc- diaminopropionic acid
methyl ester, 2 equivalents of EDC, 1 equivalent of HObt
and 3 equivalents of DIPEA were dissolved DMA. The
reaction was stirred at room temperature and monitored by
30 TLC (9/1 DCM/MeOH). Upon completion, the mixture was
concentrated *in vacuo*. The resulting oil was re suspended
in Et₂O and washed twice with 0.1 N H₂SO₄, twice with
saturated NaHCO₃, and once with brine. The organic layer
was then dried over MgSO₄, filtered and concentrated *in*
35 *vacuo*. The residue was then purified on silica gel using
5% methanol in DCM as eluent to provide pure Boc methyl
ester.

5 1 equivalent of commercially available nipecotic acid was dissolved in a 3:2 THF/H₂O solution. 1.1 equivalents of solid NaHCO₃ and 1.1 equivalents of Boc₂O were added and the mixture was stirred overnight. The reaction was concentrated to remove the THF, and the resulting aqueous
10 layer was partitioned with hexanes. The aqueous layer was then acidified to pH 2 with 1N HCl and then partitioned twice with EtOAc. The combined organic layers were dried over MgSO₄ and concentrated *in vacuo*. The resulting Boc protected nipecotic acid was used without further
15 purification.

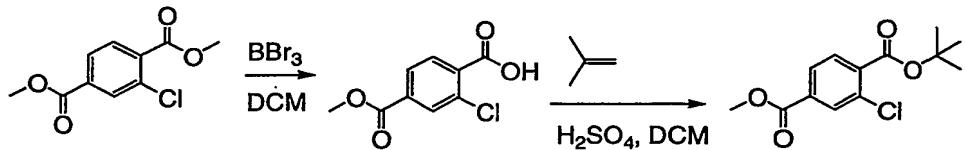
The Boc methyl ester was dissolved in a solution of TFA in DCM (1:1). After 20 minutes, the reaction was concentrated *in vacuo*. The resulting oil was dissolved in toluene and then re concentrated *in vacuo*. 1 equivalent of this amine, 2 equivalents of resulting Boc protected nipecotic acid, 2 equivalents of EDC, 1 equivalent of HObt and 3 equivalents of DIPEA were dissolved DMA. The reaction was stirred at room temperature and monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was concentrated *in vacuo*. The resulting oil was re suspended in Et₂O and washed twice with 0.1 N H₂SO₄, twice with saturated NaHCO₃, and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was then purified on silica gel using 5% methanol in DCM as eluent to provide pure product.
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25
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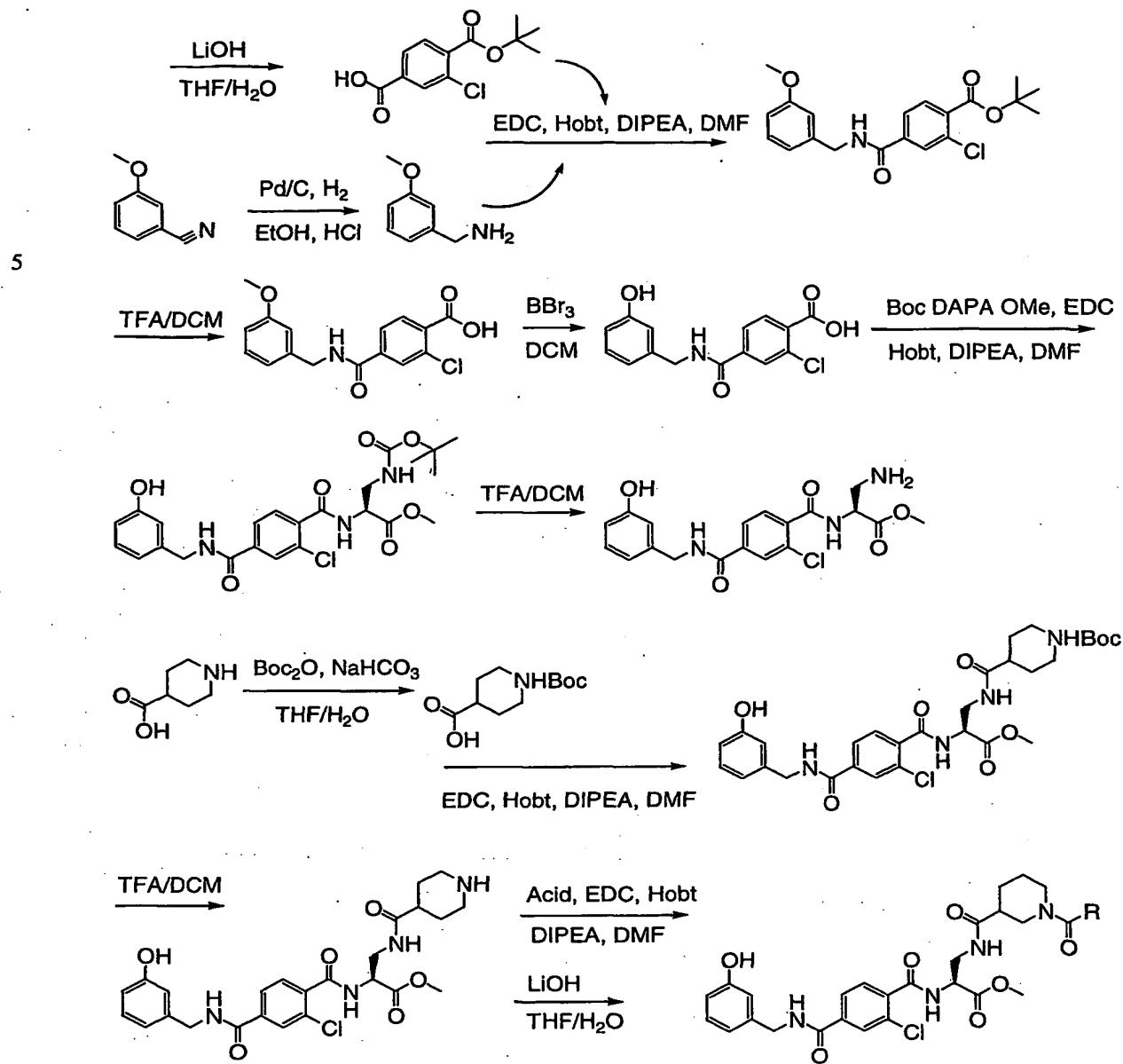
This Boc protected product was dissolved in a solution of TFA in DCM (1:1). After 20 minutes, the reaction was concentrated *in vacuo*. The resulting oil was dissolved in toluene and then concentrated *in vacuo* twice to provide pure amine. 1 equivalent of this amine, 2 equivalents of the appropriate commercially available acid (example 29;
35

5 propionic acid; example 30, acetic acid), 2 equivalents
of EDC, 1 equivalent of HObt and 3 equivalents of DIPEA
were dissolved DMA. The reaction was stirred at room
temperature and monitored by TLC (9/1 DCM/MeOH). Upon
completion, the mixture was concentrated *in vacuo*. The
10 resulting oil was re suspended in Et₂O and washed twice
with 0.1 N H₂SO₄, twice with saturated NaHCO₃, and once
with brine. The organic layer was then dried over MgSO₄,
filtered and concentrated *in vacuo*. The residue was then
purified on silica gel using 5% methanol in DCM as eluent
15 to provide pure product.

1 equivalent of the resultant methyl ester was dissolved
in THF/H₂O (3/1) and 3 equivalents of LiOH•H₂O was added.
The reaction was monitored by TLC (9/1 DCM/MeOH). Upon
20 completion, the mixture was acidified to pH 2 with 1M HCl
and then concentrated *in vacuo*. The resulting solid was
re suspended in Et₂O and washed twice with 0.1 M HCl and
once with brine. The organic layer was then dried over
MgSO₄, filtered and concentrated *in vacuo*. The resulting
25 acid was then purified by reverse phase HPLC, verified by
electrospray mass spectrometry and lyophilized to a
powder.

30 EXAMPLE 7 Synthesis of compounds 32-34





1 equivalent of dimethyl 2- chloroterephthalic acid was dissolved in DCM and cooled to -5°C in an ice/acetone bath under nitrogen. 1 equivalent of BBr_3 was added drop wise as a solution in DCM over 30 minutes. The reaction was warmed to room temperature and stirred until complete by TLC (DCM/2% HOAc/2% MeOH). The solution was poured onto ice, and the ice was allowed to melt. The mixture was then partitioned with EtOAc and concentrated *in vacuo*. This product was dissolved in H_2O with the addition

5 of saturated NaHCO₃ until the pH remained above 8. This
solution was partitioned one time with an equal volume
of DCM to remove unreacted diester. The basic solution
was acidified at 0°C. with concentrated HCl to pH = 1-
10 1.5, and precipitate was extracted twice with equal
volumes of EtOAc. The organics were partitioned once
with brine and dried over MgSO₄, filtered and concentrated
in vacuo. Product was 7:1 of the correct regioisomer by
HPLC.

15 The monoester was dissolved in DCM and transferred to a
pre-weighed Parr flask containing a stirring bar. The
flask was cooled to -5°C with a dry ice/alcohol bath
under nitrogen. Once cool, ~30 equivalents of isobutylene
was pumped into solution with stirring. 2.1 equivalents
20 of concentrated sulfuric acid was added and the flask was
sealed with a wired rubber stopper and allowed to warm to
room temperature with stirring. The solution was stirred
until clarification (1-2 days). Once the solution was
clear, it was cooled to 0°C in an ice bath. The stopper
25 was removed and the excess isobutylene was blown off with
nitrogen bubbling. Saturated NaHCO₃ was added to
neutralize the acid and the mixture was concentrated in
vacuo until no DCM remained. The solution was then
partitioned into EtOAc. The organics were partitioned
30 twice with dilute HCl, twice with saturated NaHCO₃, once
with brine, dried over MgSO₄, filtered and concentrated in
vacuo. The resulting product was used with no further
purification.

35 1 equivalent of the methyl ester was dissolved in THF/H₂O
(3/1) and 3 equivalents of LiOH·H₂O was added. The
reaction was monitored by TLC (9/1 DCM/MeOH). Upon
completion, the mixture was acidified carefully to pH 2

5 with concentrated HCl and then concentrated in vacuo to remove the THF. The resulting aqueous layer was washed twice with Et₂O and the combined organic layers were washed once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated in vacuo. The
10 benzoic acid t-butyl ester was used without further purification.

15 1 equivalent of 3-methoxybenzonitrile was placed in a Parr bottle with EtOH, 0.02 equivalents of HCl and 10% (w/w) of 10% Pd on carbon. The vessel was placed in the Parr shaker, charged with 50psi H₂, and shaken for 12 hours. The reaction filtered through a pad of celite and diluted 1:10 with Et₂O. Upon standing over night, fine white needles form. The product was filtered, washed with
20 Et₂O and dried in vacuo. The resulting amine hydrochloride salt was then used with out further purification.

25 3 equivalents of the benzoic acid t-butyl ester was coupled to 1 equivalent of the amine hydrochloride salt using 3 equivalents EDC, 1 equivalent of Hobt and 3 equivalents of DIPEA in DMA. The reaction was monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was concentrated in vacuo. The resulting oil was re suspended in Et₂O and washed twice with 0.1 N H₂SO₄, twice with
30 saturated NaHCO₃, and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated in vacuo. The product was then purified on silica get using 5% methanol in DCM as eluent to provide pure t-butyl ester.

35

The t-butyl ester was dissolved in a solution of TFA in DCM (1:1). After 20 minutes, the reaction was

5 concentrated *in vacuo*. The resulting oil was dissolved in toluene and then concentrated *in vacuo* twice.

10 The resulting compound was dissolved in DCM and cooled to -5°C in an ice/acetone bath under nitrogen. 2 equivalents of BBr₃ were added drop wise as a solution in DCM over 30 minutes. The reaction was warmed to room temperature and stirred until complete by TLC (DCM/2% HOAc/2% MeOH). The solution was poured onto ice, and the ice was allowed to melt. The mixture was then partitioned twice with EtOAc and the combined organic layers were dried over MgSO₄. The filtrate was then passed over a plug of silica gel and concentrated *in vacuo* to afford pure benzoic acid.

20 1 equivalent of the benzoic acid, 2 equivalents of commercially available D- Boc- diaminopropionic acid methyl ester, 2 equivalents of EDC, 1 equivalent of HObt and 3 equivalents of DIPEA were dissolved DMA. The reaction was stirred at room temperature and monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was concentrated *in vacuo*. The resulting oil was re suspended in Et₂O and washed twice with 0.1 N H₂SO₄, twice with saturated NaHCO₃, and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was then purified on silica gel using 5% methanol in DCM as eluent to provide pure Boc methyl ester.

35 1 equivalent of commercially available isonipecotic acid was dissolved in a 3:2 THF/H₂O solution. 1.1 equivalents of solid NaHCO₃ and 1.1 equivalents of Boc₂O were added and the mixture was stirred overnight. The reaction was concentrated to remove the THF, and the resulting aqueous layer was partitioned with hexanes. The aqueous layer was

5 then acidified to pH 2 with 1N HCl and then partitioned twice with EtOAc. The combined organic layers were dried over MgSO₄ and concentrated *in vacuo*. The resulting Boc protected isonipecotic acid was used without further purification.

10

The Boc methyl ester was dissolved in a solution of TFA in DCM (1:1). After 20 minutes, the reaction was concentrated *in vacuo*. The resulting oil was dissolved in toluene and then re concentrated *in vacuo*. 1 equivalent of this amine, 2 equivalents of resulting Boc protected isonipecotic acid, 2 equivalents of EDC, 1 equivalent of HObt and 3 equivalents of DIPEA were dissolved DMA. The reaction was stirred at room temperature and monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was concentrated *in vacuo*. The resulting oil was re suspended in Et₂O and washed twice with 0.1 N H₂SO₄, twice with saturated NaHCO₃, and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was then purified on silica gel using 5% methanol in DCM as eluent to provide pure product.

25

This Boc protected product was dissolved in a solution of TFA in DCM (1:1). After 20 minutes, the reaction was concentrated *in vacuo*. The resulting oil was dissolved in toluene and then concentrated *in vacuo* twice to provide pure amine. 1 equivalent of this amine, 2 equivalents of the appropriate commercially available acid (example 32; propionic acid; example 33, butyric acid; example 34, acetic acid), 2 equivalents of EDC, 1 equivalent of HObt and 3 equivalents of DIPEA were dissolved DMA. The reaction was stirred at room temperature and monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was concentrated *in vacuo*. The resulting oil was re suspended

5 in Et₂O and washed twice with 0.1 N H₂SO₄, twice with saturated NaHCO₃, and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was then purified on silica gel using 5% methanol in DCM as eluent to provide pure product.

10

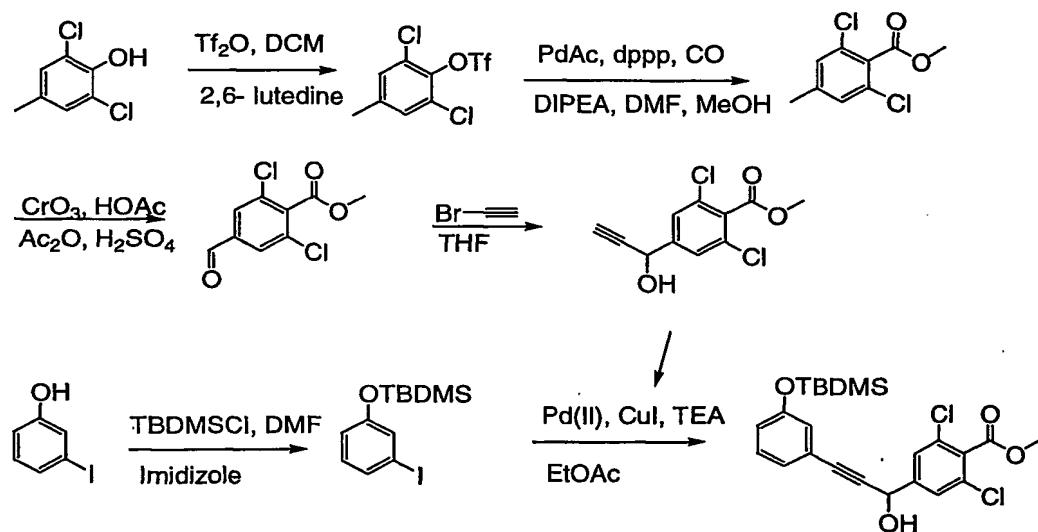
1 equivalent of the resultant methyl ester was dissolved in THF/H₂O (3/1) and 3 equivalents of LiOH·H₂O were added. The reaction was monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was acidified to pH 2 with 1M HCl and then concentrated *in vacuo*. The resulting solid was re suspended in Et₂O and washed twice with 0.1 M HCl and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*. The resulting acid was then purified by reverse phase HPLC, verified by electrospray mass spectrometry and lyophilized to a powder.

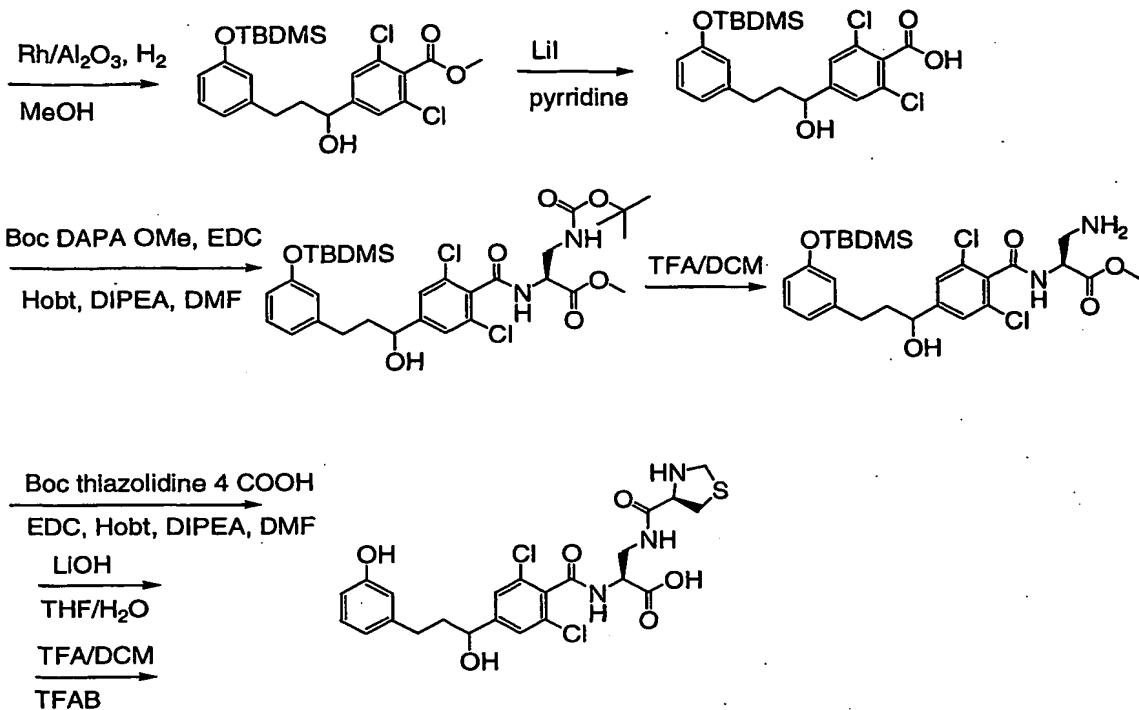
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EXAMPLE 8 Synthesis of compounds 36

25





10 1 equivalent of 2, 6-Dichloro-4-methyl phenol was dissolved in DCM containing 2.6 equivalents of 2, 6-lutidine and the mixture was cooled to -78°C. After adding 1.25 equivalents of triflic anhydride the stirring reaction was allowed to warm to room temperature overnight. The reaction was then concentrated, and the residue was partitioned between Et₂O and H₂O. The aqueous layer was extracted with Et₂O and the combined organic layers were dried over MgSO₄ and concentrated in vacuo.

15 The residue was purified by silica gel flash chromatography (9:1 hexane/Et₂O) to provide the pure triflate.

20

To a stirring solution of 1 equivalent of the triflate in a 2/1 mixture of DMF/MeOH was added 0.15 equivalents of 1, 3-bis(diphenylphosphino)-propane and 2.5 equivalents of TEA. Carbon monoxide gas was bubbled through this solution for 15 minutes, then 0.15 equivalents of Pd(OAc)₂ was added and the reaction was stirred at 70°C

5 for 5-7 hours under an atmosphere of CO (using a balloon
filled with CO). The reaction was then concentrated *in
vacuo*, and the residue was partitioned between Et₂O and
H₂O. The aqueous layer was extracted twice with Et₂O and
the combined organic layers were dried over MgSO₄,
10 filtered through a plug of silica gel and concentrated *in
vacuo*. The residue was purified by silica gel flash
chromatography (9:1:0.02 hexane/DCM/Et₂O) to provide the
pure tolyl methyl ester.

15 1 equivalent of the tolyl methyl ester was dissolved in
acetic anhydride and HOAc, then cooled in an ice-salt
bath (-5°C) before concentrated H₂SO₄ was added. A
solution of CrO₃ (2.6 equivalents) in acetic anhydride and
HOAc was added drop wise and the reaction was stirred for
20 3.5 hours at -5°C. The reaction was poured into ice H₂O
and stirred for 30 min. The mixture was extracted three
times with ethyl ether. The combined organic layers were
washed with saturated NaHCO₃ and brine, then dried over
MgSO₄ and concentrated *in vacuo* to an oil. Toluene was
added to the oil and the solution concentrated *in vacuo*
again. This was repeated to obtain a crystalline solid.
The solid was dissolved in methanol and concentrated HCl
and heated at reflux for 12 hours. The reaction was
concentrated *in vacuo* and the residue was purified by
25 silica gel flash chromatography (9:1 hexane/Et₂O) to
provide the pure aldehyde.

35 A solution of 1 equivalent of the aldehyde in THF was
cooled to -78°C and 1.1 equivalents of 0.5M
ethynylmagnesium bromide/THF was added. After stirring
the reaction at room temperature for 3 hours, it was
diluted with Et₂O and washed twice with 10% citric acid.
The combined aqueous layers were back-extracted once with

5 Et₂O. The combined organic layers were washed twice with saturated aqueous NaHCO₃, dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by silica gel flash chromatography (4:1 to 3:2 hexane/Et₂O) to provide the pure alkyne.

10 1 equivalent of 3-Iodophenol, 2.2 equivalents of t-butyldimethyl silyl chloride and 3 equivalents of imidazole were dissolved in DMF and stirred at room temperature. The reaction was monitored by TLC (9/1 DCM/MeOH). Upon reaction completion, the mixture was concentrated *in vacuo*. The resulting oil was re suspended in Et₂O and washed twice with saturated NaHCO₃, and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*. The product was then used with out further purification.

15 1 equivalent of the silyl iodide was dissolved in EtOAc and the solution was degassed by passing N₂ through a pipette and into the solution for 10 minutes. 1.25 equivalents of the alkyne was added, followed by 0.02 equivalents of dichlorobis(triphenylphosphine)-palladium(II), 0.04 equivalents of CuI and 5 equivalents TEA. The reaction was stirred for 14 hours, diluted with EtOAc, washed twice with 5% Na₂•EDTA, brine and then dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by silica gel flash chromatography (gradient elution, using Et₂O to EtOAc) to provide the pure aryl alkyne.

20 1 equivalent of the aryl alkyne was dissolved in MeOH and the solution was degassed by passing N₂ through a pipette and into the solution for 10 minutes. The 5% Rh/Al₂O₃ was added, one balloon-full of hydrogen was passed through the solution, and the reaction was stirred under an

5 atmosphere of H₂ (using a balloon) for 7 hours, after which the reaction was filtered through a pad of celite and concentrated *in vacuo*. The residue was purified by silica gel flash chromatography (gradient elution, using Et₂O to EtOAc) to provide the pure product.

10 2.3 equivalents of lithium iodide was added to 1 equivalent of the methyl ester in pyridine, and the mixture heated at reflux for 8 hours. The reaction was concentrated *in vacuo* and the residue was partitioned
15 between EtOAc and 1N HCl. The aqueous layer was extracted three times with EtOAc, and the combined organic layers were washed with 1M NaHCO₃, dried over MgSO₄ and concentrated *in vacuo*. The residue was dissolved in NMM and the solution concentrated *in vacuo*. The residue was taken up in DCM and then washed three times with 1N HCl.
20 The organic layer was dried over MgSO₄ and concentrated *in vacuo* to provide the benzoic acid in high enough purity to be used without further purification.

25 1 equivalent of the acid, 2 equivalents of commercially available β -Boc- diaminopropionic acid methyl ester, 2 equivalents of EDC, 1 equivalent of Hobt and 3 equivalents of DIPEA were dissolved DMA. The reaction was stirred at room temperature and monitored by TLC (9/1
30 DCM/MeOH). Upon completion, the mixture was concentrated *in vacuo*. The resulting oil was re suspended in Et₂O and washed twice with 0.1 N H₂SO₄, twice with saturated NaHCO₃, and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*. The
35 residue was then purified on silica gel using 5% methanol in DCM as eluent to provide pure methyl ester.

5 The Boc protected amine was dissolved in a solution of TFA in DCM (1:1). After 20 minutes, the reaction was concentrated *in vacuo*. The resulting oil was dissolved in toluene and then reconcentrated *in vacuo*. 1 equivalent of this amine, 2 equivalents of Boc-L-thiazolidine-4-carboxylic acid, 2 equivalents of EDC, 1 equivalent of HObt and 3 equivalents of DIPEA were dissolved DMA. The reaction was stirred at room temperature and monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was concentrated *in vacuo*. The resulting oil was re suspended in Et₂O and washed twice with 0.1 N H₂SO₄, twice with saturated NaHCO₃, and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was then purified on silica gel using 5% methanol in DCM as eluent to provide pure methyl ester.

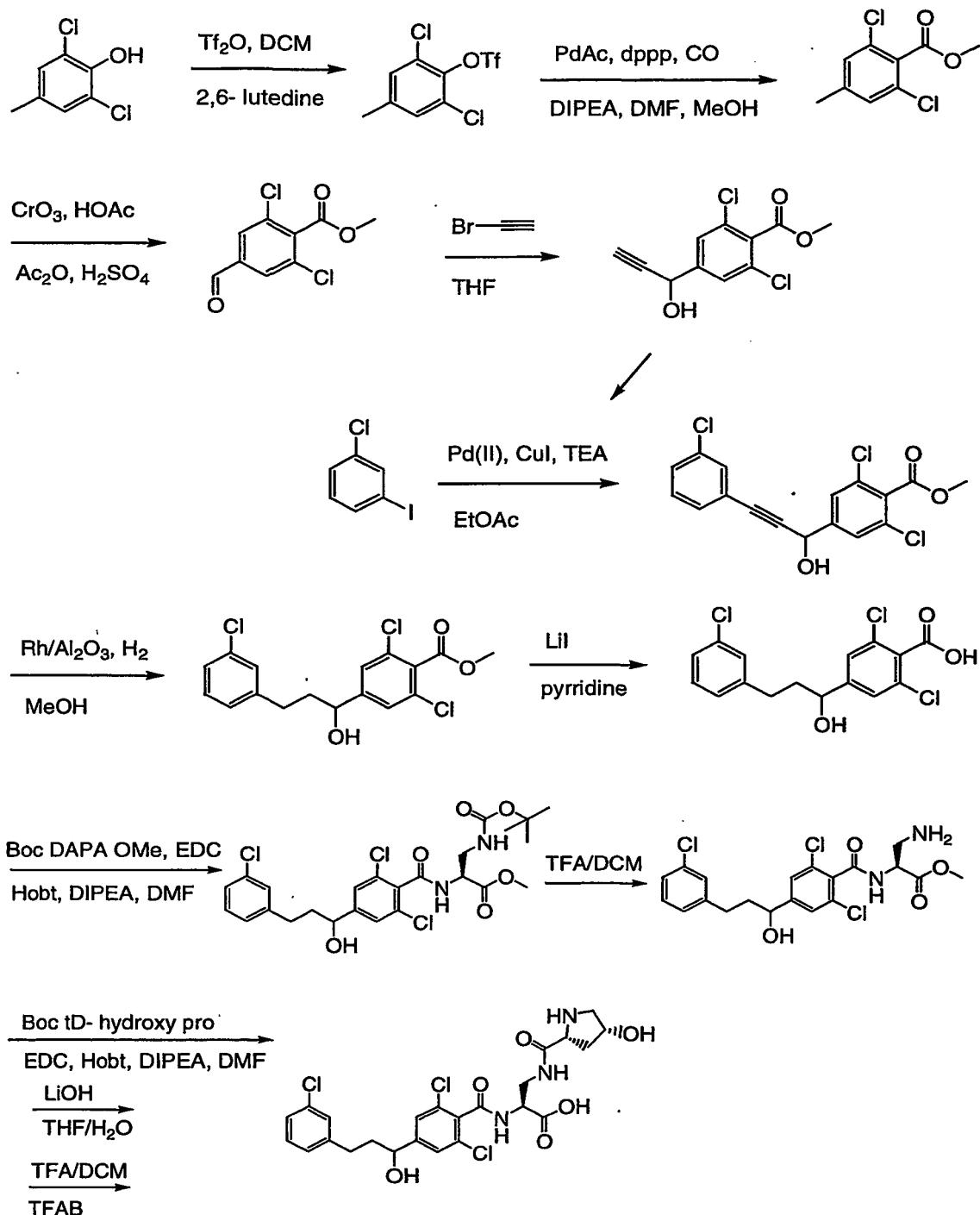
1 equivalent of the resultant methyl ester was dissolved in THF/H₂O (3/1) and 3 equivalents of LiOH·H₂O was added. The reaction was monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was acidified to pH 2 with 1M HCl and then concentrated *in vacuo*. The resulting solid was re suspended in Et₂O and washed twice with 0.1 M HCl and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*.

30 The Boc, silyl residue was dissolved in a solution of TFA in DCM (1:1) with 3 equivalents of TBAF. After 20 minutes, the reaction was concentrated *in vacuo*. The resulting oil was dissolved in toluene and then reconcentrated *in vacuo*. The resulting acid was then purified by reverse phase HPLC, verified by electrospray mass spectrometry and lyophilized to a powder.

5

EXAMPLE 9

Synthesis of compounds 37



15

1 equivalent of 2, 6-Dichloro-4-methyl phenol was dissolved in DCM containing 2.6 equivalents of 2, 6-lutidine and the mixture was cooled to -78°C. After

5 adding 1.25 equivalents of triflic anhydride the stirring reaction was allowed to warm to room temperature overnight. The reaction was then concentrated, and the residue was partitioned between Et₂O and H₂O. The aqueous layer was extracted with Et₂O and the combined organic 10 layers were dried over MgSO₄ and concentrated in vacuo. The residue was purified by silica gel flash chromatography (9:1 hexane/Et₂O) to provide the pure triflate.

15 To a stirring solution of 1 equivalent of the triflate in a 2/1 mixture of DMF/MeOH was added 0.15 equivalents of 1, 3-bis(diphenylphosphino)-propane and 2.5 equivalents of TEA. Carbon monoxide gas was bubbled through this solution for 15 minutes, then 0.15 equivalents of 20 Pd(OAc)₂ was added and the reaction was stirred at 70°C for 5-7 hours under an atmosphere of CO (using a balloon filled with CO). The reaction was then concentrated in vacuo, and the residue was partitioned between Et₂O and H₂O. The aqueous layer was extracted twice with Et₂O and 25 the combined organic layers were dried over MgSO₄, filtered through a plug of silica gel and concentrated in vacuo. The residue was purified by silica gel flash chromatography (9:1:0.02 hexane/DCM/Et₂O) to provide the pure tolyl methyl ester.

30 1 equivalent of the tolyl methyl ester was dissolved in acetic anhydride and HOAc, then cooled in an ice-salt bath (-5°C) before concentrated H₂SO₄ was added. A solution of CrO₃ (2.6 equivalents) in acetic anhydride and 35 HOAc was added drop wise and the reaction was stirred for 3.5 hours at -5°C. The reaction was poured into ice H₂O and stirred for 30 min. The mixture was extracted three times with ethyl ether. The combined organic layers were

5 washed with saturated NaHCO₃ and brine, then dried over MgSO₄ and concentrated *in vacuo* to an oil. Toluene was added to the oil and the solution concentrated *in vacuo* again. This was repeated to obtain a crystalline solid.
10 The solid was dissolved in methanol and concentrated HCl and heated at reflux for 12 hours. The reaction was concentrated *in vacuo* and the residue was purified by silica gel flash chromatography (9:1 hexane/Et₂O) to provide the pure aldehyde.

15 A solution of 1 equivalent of the aldehyde in THF was cooled to -78°C and 1.1 equivalents of 0.5M ethynylmagnesium bromide/THF was added. After stirring the reaction at room temperature for 3 hours, it was diluted with Et₂O and washed twice with 10% citric acid.
20 The combined aqueous layers were back-extracted once with Et₂O. The combined organic layers were washed twice with saturated aqueous NaHCO₃, dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by silica gel flash chromatography (4:1 to 3:2 hexane/Et₂O) to provide the pure alkyne.
25

1 1 equivalent of 1-chloro-3-iodobenzene was dissolved in EtOAc and the solution was degassed by passing N₂ through a pipette and into the solution for 10 minutes. 1.25 equivalents of the alkyne was added, followed by 0.02 equivalents of dichlorobis(triphenylphosphine)palladium(II), 0.04 equivalents of CuI and 5 equivalents TEA. The reaction was stirred for 14 hours, diluted with EtOAc, washed twice with 5% Na₂·EDTA, brine and then dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by silica gel flash chromatography (gradient elution, using Et₂O to EtOAc) to provide the pure aryl alkyne.

5 1 equivalent of the aryl alkyne was dissolved in MeOH and
the solution was degassed by passing N₂ through a pipette
and into the solution for 10 minutes. The 5% Rh/Al₂O₃ was
added, one balloon-full of hydrogen was passed through
the solution, and the reaction was stirred under an
10 atmosphere of H₂ (using a balloon) for 7 hours, after
which the reaction was filtered through a pad of celite
and concentrated *in vacuo*. The residue was purified by
silica gel flash chromatography (gradient elution, using
Et₂O to EtOAc) to provide the pure product.

15 2.3 equivalents of lithium iodide was added to 1
equivalent of the methyl ester in pyridine, and the
mixture heated at reflux for 8 hours. The reaction was
concentrated *in vacuo* and the residue was partitioned
20 between EtOAc and 1N HCl. The aqueous layer was extracted
three times with EtOAc, and the combined organic layers
were washed with 1M NaHCO₃, dried over MgSO₄ and
concentrated *in vacuo*. The residue was dissolved in NMM
and the solution concentrated *in vacuo*. The residue was
25 taken up in DCM and then washed three times with 1N HCl.
The organic layer was dried over MgSO₄ and concentrated *in
vacuo* to provide the benzoic acid in high enough purity
to be used without further purification.

30 1 equivalent of the acid, 2 equivalents of commercially
available β - Boc- diaminopropionic acid methyl ester, 2
equivalents of EDC, 1 equivalent of Hobt and 3
equivalents of DIPEA were dissolved DMA. The reaction was
stirred at room temperature and monitored by TLC (9/1
35 DCM/MeOH). Upon completion, the mixture was concentrated
in vacuo. The resulting oil was re suspended in Et₂O and
washed twice with 0.1 N H₂SO₄, twice with saturated
NaHCO₃, and once with brine. The organic layer was then

5 dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was then purified on silica gel using 5% methanol in DCM as eluent to provide pure methyl ester.

10 1 equivalent of commercially available D-hydroxy proline was dissolved in a 3:2 THF/H₂O solution. 1.1 equivalents of solid NaHCO₃ and 1.1 equivalents of Boc₂O were added and the mixture was stirred overnight. The reaction was concentrated to remove the THF, and the resulting aqueous
15 layer was partitioned with hexanes. The aqueous layer was then acidified to pH 2 with 1N HCl and then partitioned twice with EtOAc. The combined organic layers were dried over MgSO₄ and concentrated *in vacuo*. The resulting N-Boc-D-hydroxy proline was used without further purification.

20 The Boc protected amine was dissolved in a solution of TFA in DCM (1:1). After 20 minutes, the reaction was concentrated *in vacuo*. The resulting oil was dissolved in toluene and then reconcentrated *in vacuo*. 1 equivalent of
25 this amine, 2 equivalents of Boc-D-hydroxy proline, 2 equivalents of EDC, 1 equivalent of HObt and 3 equivalents of DIPEA were dissolved DMA. The reaction was stirred at room temperature and monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was concentrated
30 *in vacuo*. The resulting oil was re suspended in Et₂O and washed twice with 0.1 N H₂SO₄, twice with saturated NaHCO₃, and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was then purified on silica gel using 5% methanol in DCM as eluent to provide pure methyl ester.

35 1 equivalent of the resultant methyl ester was dissolved in THF/H₂O (3/1) and 3 equivalents of LiOH•H₂O was added.

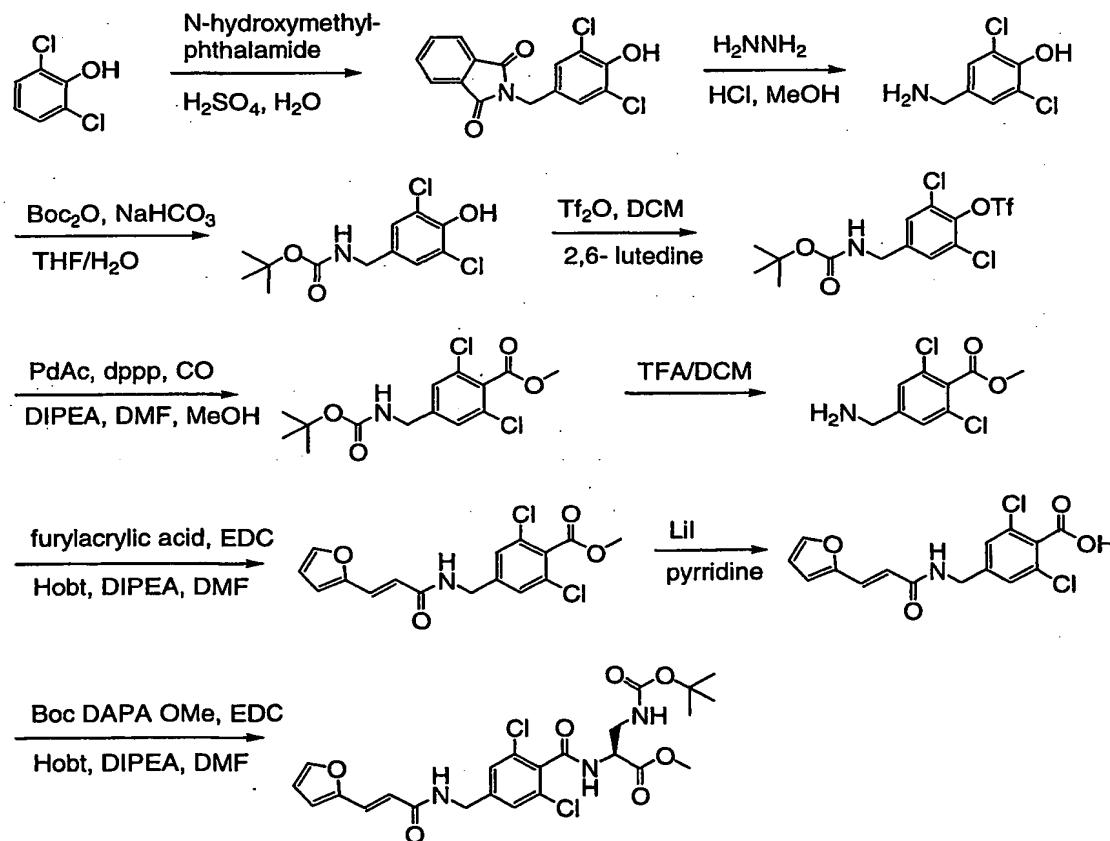
5 The reaction was monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was acidified to pH 2 with 1M HCl and then concentrated *in vacuo*. The resulting solid was re suspended in Et₂O and washed twice with 0.1 M HCl and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*. The Boc, silyl residue was dissolved in a solution of TFA in DCM (1:1). After 20 minutes, the reaction was concentrated *in vacuo*. The resulting oil was dissolved in toluene and then reconcentrated *in vacuo*. The resulting acid was then purified by reverse phase HPLC, verified by electrospray mass spectrometry and lyophilized to a powder.

10

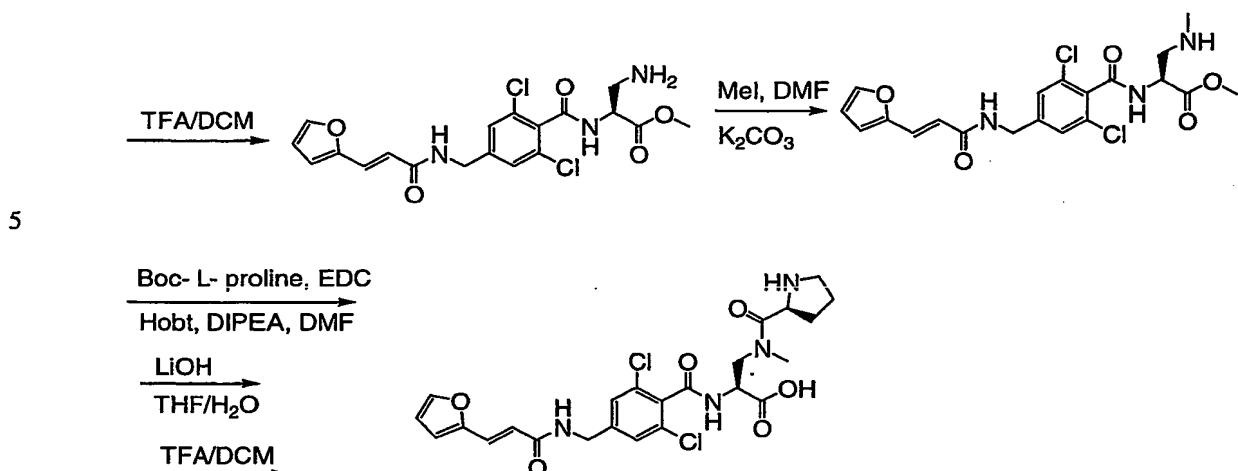
15

EXAMPLE 10 Synthesis of compound 35

20



25



A round bottom flask was equipped with an efficient overhead stirrer and charged with concentrated H₂SO₄ (2.7 x volume of H₂O) and H₂O and cooled to ~-5°C with an ethanol/ice bath. Once cool, 1 equivalent 2.6 dichloro phenol and 1 equivalent of N-(hydroxymethyl)phthalimide were added with vigorous stirring. The reaction was kept cool for 4 hours and then allowed to warm to room temperature overnight with constant stirring. The reaction generally proceeds to a point where there was just a solid in the round bottom flask. At this point EtOAc and H₂O were added and stirred into the solid. Large chunks were broken up and then the precipitate was filtered and washed with more EtOAc and H₂O. The product was then used without further purification after drying overnight under vacuum.

1 equivalent of the dry product and methanol (22.5ml x #g of starting material) was added to a round bottom flask equipped with a H₂O condenser and stirring bar. 1.2 equivalents of hydrazine mono hydrate was added and the mixture refluxed for 4 hours. After cooling to room temperature, concentrated HCl (4.5ml x #g of starting material) was carefully added. Upon completion of the

5 addition, the mixture was refluxed overnight (> 8 hours). The reaction was cooled to 0°C and the precipitated by-product was removed by filtration. The filtrate was then concentrated *in vacuo*.

10 The crude amine residue was dissolved in a 3:2 THF/H₂O solution. 1.1 equivalents of solid NaHCO₃ and 1.1 equivalents of Boc₂O were added and the mixture was stirred overnight. The reaction was concentrated, and the residue was partitioned between H₂O and Et₂O. The aqueous 15 layer was extracted with Et₂O and the combined organic layers were dried over MgSO₄ and concentrated *in vacuo* to a solid. Recrystallization from hot methanol and H₂O provided pure product.

20 1 equivalent of the Boc protected amine and 1.5 equivalents of 2, 6-lutidine was dissolved, with mild heating if necessary, in DCM in a round bottom flask. Once the starting material has completely dissolved, the mixture was cooled to -78°C under N₂ with a dry ice 25 ethanol bath. Once cool, 2.5 equivalents of triflic anhydride was added and the reaction was allowed to slowly come to room temperature with stirring. The reaction was monitored by TLC and was generally done in 4 hours. Upon completion, the reaction was concentrated *in vacuo* and the residue partitioned between EtOAc and H₂O. 30 The organic layer was washed twice with 0.1N H₂SO₄, twice with saturated NaHCO₃, once with brine, dried over MgSO₄ and concentrated *in vacuo*. The residue was then purified 35 on silica gel using DCM as eluent to provide pure triflate.

1 equivalent of triflate was dissolved in DMF and MeOH in the glass insert of a high pressure Parr bomb. The

5 starting material was then degassed while stirring with CO for 10 minutes. 0.15 equivalents palladium(II) acetate and 0.15 equivalents of 1, 3- bis(diphenylphosphino) propane were then added and the mixture was then degassed while stirring with CO for another 10 minutes at which
10 time 2.5 equivalents of diisopropyl ethyl amine was added. After properly assembling the bomb, it was charged with 300psi CO gas and heated to 70°C with stirring overnight. The bomb was then cooled and vented. The mixture was transferred to a round bottom flask and
15 concentrated *in vacuo*. The residue was then purified on silica gel using DCM with 1% acetone and 1% TEA as eluent to provide pure methyl ester.

20 The Boc protected amine was dissolved in a solution of TFA in DCM (1:1). After 20 minutes, the reaction was concentrated *in vacuo*. The resulting oil was dissolved in toluene and then reconcentrated *in vacuo*. The TFA salt of the amine was dissolved in Et₂O and washed twice with a 10% solution of K₂CO₃ in H₂O and once with brine. The
25 organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*.

30 1 equivalent of the free based amine, 3 equivalents of furylacrylic acid, 3 equivalents of EDC and 1 equivalent of Hobt were dissolved DMA. The reaction was stirred at room temperature and monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was concentrated *in vacuo*. The resulting oil was re suspended in Et₂O and washed twice with 0.1 N H₂SO₄, twice with saturated NaHCO₃, and once with brine. The organic layer was then dried over
35 MgSO₄, filtered and concentrated *in vacuo*. The residue was then purified on silica get using 5% methanol in DCM as eluent to provide pure methyl ester.

5

2.3 equivalents of lithium iodide was added to 1 equivalent of the methyl ester in pyridine, and the mixture heated at reflux for 8 hours. The reaction was concentrated *in vacuo* and the residue was partitioned
10 between EtOAc and 1N HCl. The aqueous layer was extracted three times with EtOAc, and the combined organic layers were washed with 1M NaHCO₃, dried over MgSO₄ and concentrated *in vacuo*. The residue was dissolved in NMM and the solution concentrated *in vacuo*. The residue was
15 taken up in DCM and then washed three times with 1N HCl. The organic layer was dried over MgSO₄ and concentrated *in vacuo* to provide the benzoic acid in high enough purity to be used without further purification.

20 1 equivalent of the acid, 2 equivalents of commercially available β -Boc-diaminopropionic acid methyl ester, 2 equivalents of EDC, 1 equivalent of Hobt and 3 equivalents of DIPEA were dissolved DMA. The reaction was stirred at room temperature and monitored by TLC (9/1
25 DCM/MeOH). Upon completion, the mixture was concentrated *in vacuo*. The resulting oil was re suspended in Et₂O and washed twice with 0.1 N H₂SO₄, twice with saturated NaHCO₃, and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*. The
30 residue was then purified on silica gel using 5% methanol in DCM as eluent to provide pure methyl ester.

The Boc protected amine was dissolved in a solution of TFA in DCM (1:1). After 20 minutes, the reaction was concentrated *in vacuo*. The resulting oil was dissolved in toluene and then re concentrated *in vacuo*.

5 To 1 equivalent of this amine was added 1.05 equivalents
of methyl iodide and 2.1 equivalents potassium carbonate
in DMF. The reaction was stirred at room temperature and
followed by TLC (9/1 DCM/MeOH). Upon completion of the
reaction, it was diluted with EtOAc and H₂O. The aqueous
10 layer was partitioned again with EtOAc and the combined
organic layers washed with brine, dried over MgSO₄ and
concentrated *in vacuo*.

15 1 equivalent of this amine, 2 equivalents of Boc-L-
thiazolidine-4-carboxylic acid, 2 equivalents of EDC, 1
equivalent of Hobt and 3 equivalents of DIPEA were
dissolved DMA. The reaction was stirred at room
temperature and monitored by TLC (9/1 DCM/MeOH). Upon
completion, the mixture was concentrated *in vacuo*. The
20 resulting oil was re suspended in Et₂O and washed twice
with 0.1 N H₂SO₄, twice with saturated NaHCO₃, and once
with brine. The organic layer was then dried over MgSO₄,
filtered and concentrated *in vacuo*. The residue was then
purified on silica gel using 5% methanol in DCM as eluent
25 to provide pure methyl ester.

30 1 equivalent of the resultant methyl ester was dissolved
in THF/H₂O (3/1) and 3 equivalents of LiOH•H₂O was added.
The reaction was monitored by TLC (9/1 DCM/MeOH). Upon
completion, the mixture was acidified to pH 2 with 1M HCl
and then concentrated *in vacuo*. The resulting solid was
re suspended in Et₂O and washed twice with 0.1 M HCl and
once with brine. The organic layer was then dried over
MgSO₄, filtered and concentrated *in vacuo*.

35 The residue was dissolved in a solution of TFA in DCM
(1:1). After 20 minutes, the reaction was concentrated *in
vacuo*. The resulting oil was dissolved in toluene and
then re concentrated *in vacuo*. The resulting acid was

5 then purified by reverse phase HPLC, verified by
electrospray mass spectrometry and lyophilized to a
powder.

10

EXAMPLE 11 PLM2 Antibody Capture LFA-1:ICAM-1 Assay

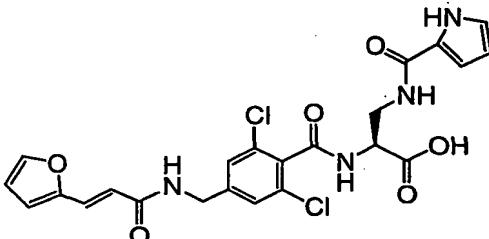
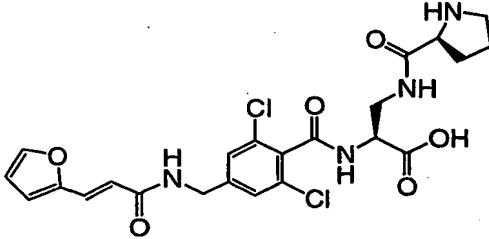
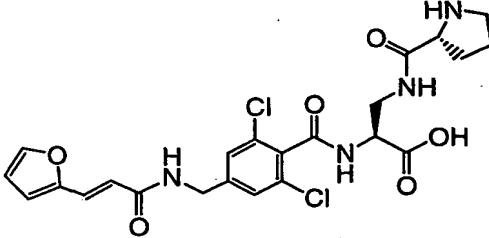
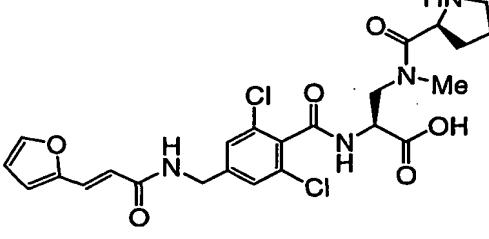
A non-function blocking monoclonal antibody against human CD18, PLM-2 (as described by Hildreth, et al., *Molecular Immunology*, Vol. 26, No. 9, pp. 883-895, 1989), is diluted to 5 μ g/ml in PBS and 96-well flat-bottomed plates are coated with 100 μ l/well overnight at 4°C. The plates are blocked with 0.5% BSA in assay buffer (0.02M Hepes, 0.15M NaCl, and 1mM MnCl₂) 1h at room temperature.
Plates are washed with 50mM Tris pH 7.5, 0.1M NaCl, 0.05% Tween 20 and 1mM MnCl₂. Purified full-length recombinant human LFA-1 protein is diluted to 2 μ g/ml in assay buffer and 100 μ l/well is added to plates and incubated 1h at 37°C. Plates are washed 3X. 50 μ l/well inhibitors, appropriately diluted in assay buffer, are added to a 2X final concentration and incubated for 30' at 37°C. 50 μ l/well of purified recombinant human 5-domain ICAM-Ig, diluted to 161ng/ml (for a final concentration of 80ng/ml) in assay buffer, is added and incubated 2h at 37°C. Plates are washed and bound ICAM-Ig is detected with Goat anti-HuIgG(Fc)-HRP for 1h at room temperature. Plates are washed and developed with 100 μ l/well TMB substrate for 5-10' at room temperature. Colorimetric development is stopped with 100 μ l/well 1M H₃PO₄ and read at 450nM on a platereader. Results of the PLM2 assay are shown in tables 1-4 below.

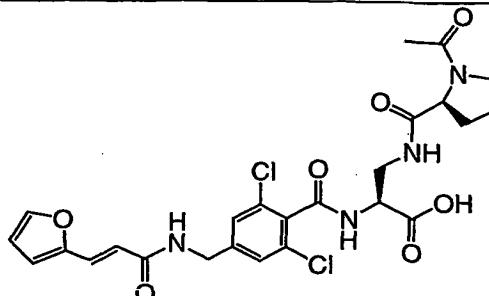
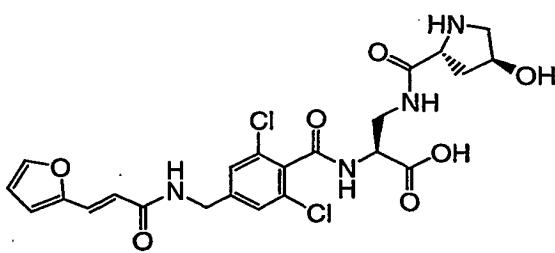
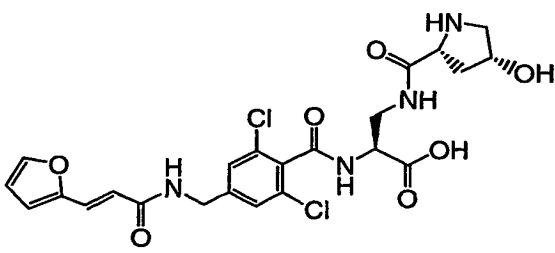
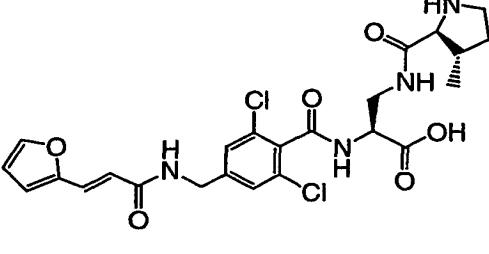
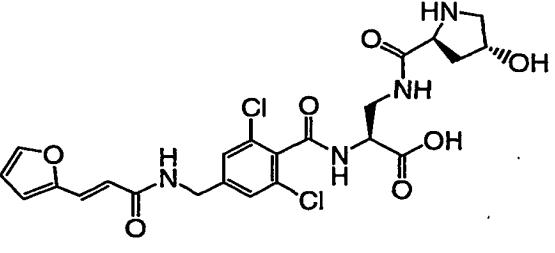
5 EXAMPLE 12 serum/plasma protein binding

Binding of test compounds was performed according to procedures described in Borga et al (Journal of Pharmacokinetics & Biopharmaceutics, 1997, 25(1):63-77) and Godolphin et al (Therapeutic drug monitoring, 1983, 5:319-23). Duplicate samples of 10 μ L of test compound stock solution (1 μ g/ μ L) was spiked into 1 mL of either buffer or serum/plasma adjusted to pH 7.4 using CO₂ at room temperature. Samples were equilibrated by incubating vials in a water bath with shaker at 37°C for 15 minutes. 200 μ L of the buffer spiked sample was saved as prefiltrate. 800 μ L of buffer spiked samples and 1 mL of serum spiked samples were centrifuged at 1500 g, 37°C, for 30 minutes in a Centrifree ultrafiltration device (Amicon Inc.). Pre and post-filtrates were then analyzed by LC/MS-MS and percent binding of test compound to serum/plasma protein was determined from the post and prefiltrates accounting for any non-specific binding determined from the buffer control.

25 Compounds of the invention incorporating a non-aromatic ring at substituent Cy surprisingly exhibit low serum plasma protein binding characteristics which is advantageous for maintaining therapeutically relevant serum levels. As illustrated in tables 1-4, reference compounds (ref) having an aromatic ring at substituent Cy consistently show higher % plasma protein binding compared to the equivalent compound of the invention having a non-aromatic ring.

5 table 1

cmpd no.	LFA-1 PLM2 IC ₅₀ (μM)	Mac-1 IC ₅₀ (μM)	% plasma protein binding	structure
ref	0.071		98.3	
4	0.004		82.9	
5	0.008		83.1	
35	0.009		51.36	

17	0.003		84.61	
10	0.003		65.91	
12	0.002		79.48	
13	0.004		77.58	
14	0.002		72.60	

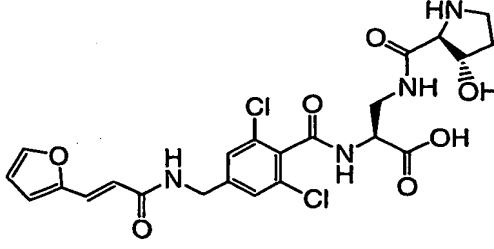
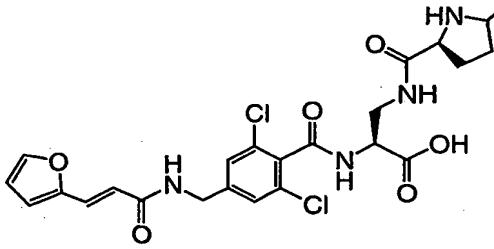
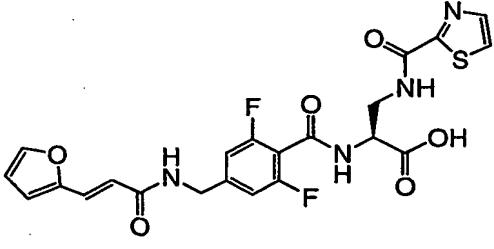
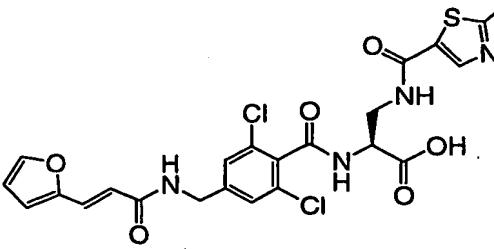
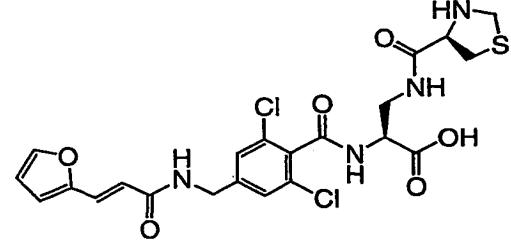
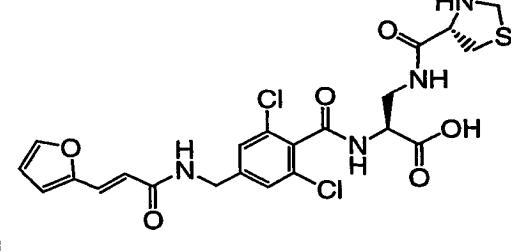
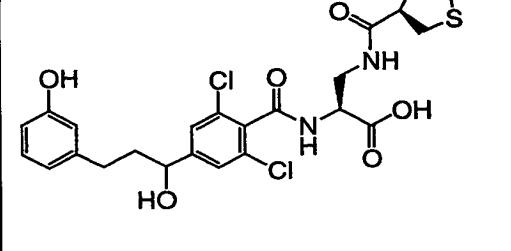
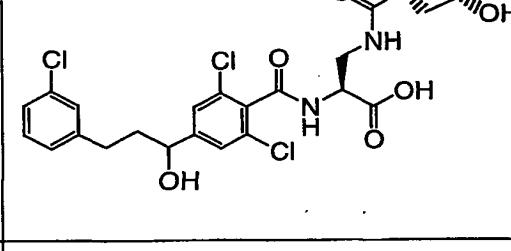
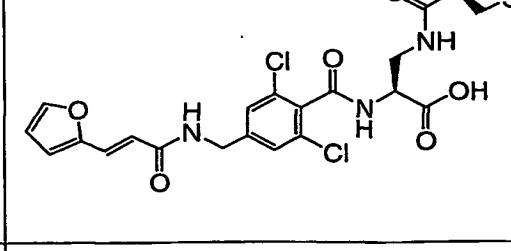
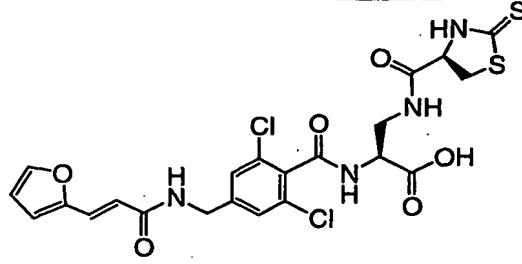
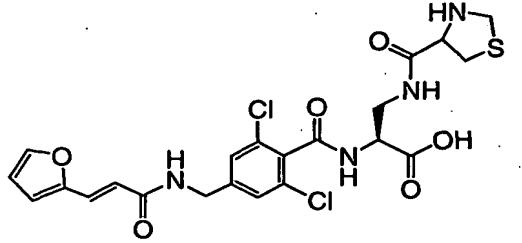
41	0.003		84.83	
44	0.002		82.97	

table 2

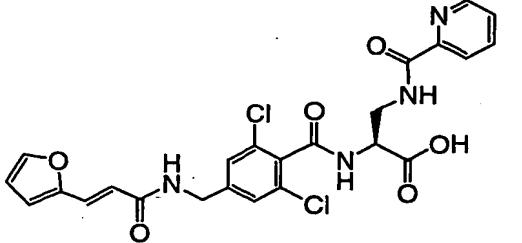
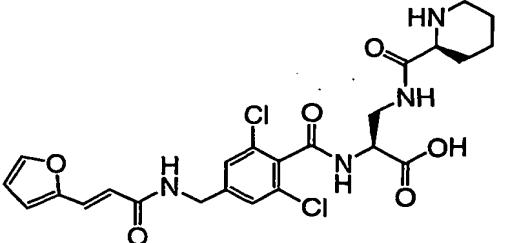
cmpd no.	LFA-1 PLM2 IC ₅₀ (μM)	Mac-1 IC ₅₀ (μM)	% plasma protein binding	structure
ref	0.005		98.12	
ref	0.004	161	99.5	

6	0.007	2509	95.43	
15	0.004		92.51	
36	0.002	65	92.84	
37		35.54	93.19	
38	0.012	7609	93.29	

40	0.002	1427	96.93	
42	0.003		91.4	

5

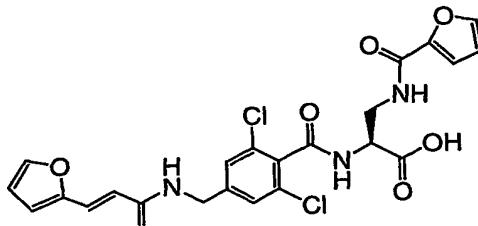
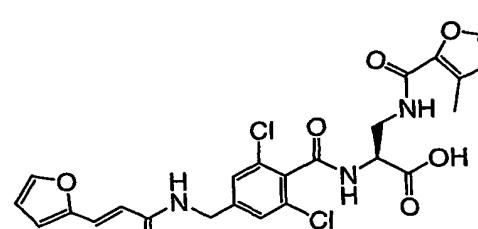
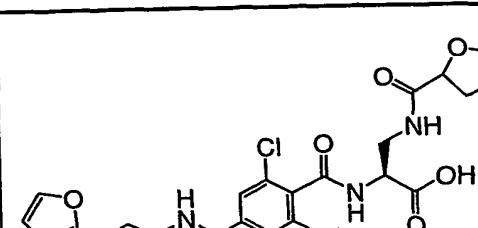
table 3

cmpd no.	LFA-1 PLM2 IC_{50} (μ M)	Mac-1 IC_{50} (μ M)	% plasma protein binding	structure
ref	0.015		99.4	
9	0.002		77.17	

3	0.011		80.8	<p>The structure shows a complex molecule with a furylvinyl group, a dichlorophenyl ring, a tritylbenzyl group, and a 2-hydroxy-3-methyl-1-azabicyclo[3.1.0]hex-3-en-3-yl group.</p>
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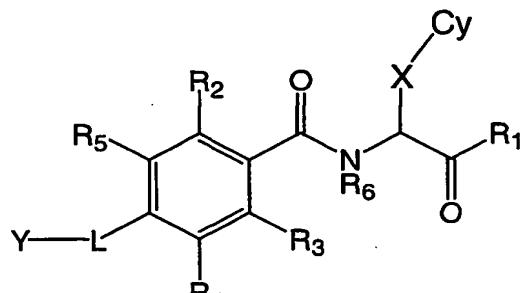
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table 4

cmpd no.	LFA-1 PLM2 IC_{50} (μ M)	Mac-1 IC_{50} (μ M)	% plasma protein binding	structure
ref			99.2	
ref	0.002	1683	99.70	
51	0.005	2362	92.8	

5 WE CLAIM:

1. A compound of formula (I)



(I)

wherein

10 Cy is a non-aromatic carbocycle or heterocycle optionally substituted with hydroxyl, mercapto, thioalkyl, halogen, oxo, thio, amino, aminoalkyl, amidine, guanidine, nitro, alkyl, alkoxy or acyl;

15 X is a divalent hydrocarbon chain optionally substituted with hydroxyl, mercapto, halogen, amino, aminoalkyl, nitro, oxo or thio and optionally interrupted with N, O, S, SO or SO₂;

20 Y is a carbocycle or heterocycle optionally substituted with hydroxyl, mercapto, halogen, oxo, thio, thioalkyl, amino, aminoalkyl, carbocycle or heterocycle ring, hydrocarbon, a halo-substituted hydrocarbon, amino, amidine, guanidine, cyano, nitro, alkoxy or acyl;

25 L is a bond or a divalent hydrocarbon chain optionally substituted hydroxyl, halogen, oxo or thio and optionally interrupted with N, O, S, SO or SO₂ or an amino acid residue; less than 3 or 5 atoms

30 R₁ is H, OH, amino, O-carbocycle or alkoxy optionally substituted with amino, a carbocycle or heterocycle;

5 R₂₋₅ are independently H, hydroxyl, mercapto, halogen, cyano, amino, amidine, guanidine, nitro or alkoxy; or R₃ and R₄ together form a fused carbocycle or heterocycle optionally substituted with hydroxyl, halogen, oxo, thio, amino, amidine, guanidine or alkoxy;

10 R₆ is H or a hydrocarbon chain optionally substituted with a carbocycle or a heterocycle; and salts, solvates and hydrates thereof;

15 with the proviso that when Y is phenyl, R₂, R₄ and R₅ are H, R₃ is Cl and R₁ is OH then X is other than cyclohexyl.

2. A compound according to claim 1, wherein Cy is a 5- or 6-member non-aromatic heterocycle optionally substituted with hydroxyl, mercapto, thioalkyl, halogen, oxo, thio, amino, aminoalkyl, amidine, guanidine, nitro, alkyl, alkoxy or acyl.

20 3. A compound according to claim 2, wherein said heterocycle comprises one or two heteroatoms and is optionally substituted with hydroxyl, oxo, mercapto, thio, alkyl or alkanoyl.

25 4. A compound according to claim 3, wherein said heterocycle is selected from the group consisting of piperidine, piperazine, morpholine, tetrahydrofuran, tetrahydrothiophene, oxazolidine, cyclopropa-pyrrolidine and thiazolidine optionally substituted with hydroxy, oxo, mercapto, thio, alkyl or alkanoyl.

30 35 5. A compound according to claim 4, wherein said heterocycle is selected from the group consisting of

5 piperidine, piperazine, morpholine, tetrahydrofuran,
tetrahydrothiophene, oxazolidine, thiazolidine
optionally substituted with hydroxy, oxo, mercapto,
thio, alkyl or alkanoyl.

10 6. A compound according to claim 1, wherein Cy is a 3-6
member carbocycle optionally substituted with
hydroxyl, mercapto, halogen, oxo, thio, amino,
amidine, guanidine, alkyl, alkoxy or acyl.

15 7. A compound according to claim 6, wherein said
carbocycle is partially unsaturated.

20 8. A compound according to claim 7, wherein Cy is
cyclopropyl, cyclypropenyl, cyclobutyl, cyclbutenyl,
cyclopentyl, cyclopentenyl cyclohexyl or
cyclohexenyl.

25 9. A compound according to claim 1, wherein X is a C₁₋₅
divalent hydrocarbon optionally having one or more
carbon atoms replaced with N, O, S, SO or SO₂ and
optionally being substituted with hydroxyl, oxo or
thio.

30 10. A compound according to claim 1, wherein X is -CH₂-
NR₆-C(O)- wherein the carbonyl -C(O)- portion thereof
is covalently bound to Cy and R₆ is H or alkyl.

35 11. A compound according to claim 1, wherein Y is a
carbocycle or heterocycle optionally substituted
with hydroxyl or halogen.

12. A compound according to claim 11, wherein Y is
furan-2-yl, thiophene-2-yl or phenyl, wherein said

5 phenyl is optionally substituted with halogen or hydroxyl.

10

13. A compound according to claim 1, wherein L is a divalent hydrocarbon optionally having one or more carbon atoms replaced with N, O, S, SO or SO₂ and optionally being substituted with hydroxyl, halogen oxo or thio; or three carbon atoms of the hydrocarbon are replaced with an amino acid residue.

15

14. A compound according to claim 13, wherein L is -CH=CH-C(O)-NR₆-CH₂-, -CH₂-NR₆-C(O)-, -C(O)-N₆-CH₂-, -CH(OH)-(CH₂)₂-, -(CH₂)₂-CH(OH)-, -(CH₂)₃-, -C(O)-NR₆-CH(R₇)-C(O)-NR₆-, -NR₆-C(O)-CH(R₇)-NR₆-C(O)-, -CH(OH)-CH₂-O- or -CH(OH)-CF₂-CH₂- wherein each R₆ is independently H or alkyl and R₇ is an amino acid side chain.

20

15. A compound according to claim 14, wherein R₁ is H, OH, amino, O-carbocycle or alkoxy optionally substituted with a carbocycle.

25

16. A compound according to claim 15, wherein R₁ is H or C₁₋₄ alkyloxy.

30

17. A compound according to claim 1, wherein at least one of R₂ and R₃ is halogen and the other is H or halogen.

35

18. A compound according to claim 17, wherein R₂ and R₃ are both Cl.

19. A compound according to claim 18, wherein R₄ and R₅ are both H.

5

20. A pharmaceutical composition comprising a compound according to claim 1 with a pharmaceutically acceptable adjuvant, diluent or carrier.
- 10 21. A method of inhibiting binding of a LFA-1 to a protein ligand comprising contacting LFA-1 with a compound of claim 1.
22. A method of treating a disease or condition mediated by LFA-1 in a mammal comprising administering to said mammal an effective amount of a compound according to claim 1.
23. A method according to claim 23, wherein said disease or condition is arthritis, psoriasis, organ transplant rejection, asthma, and inflammatory bowel disease
- 15 23. A method of inhibiting an inflammatory disease or condition in a mammal comprising administering to said mammal an effective amount of a compound according to claim 1.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/44203

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07D405/12 C07D417/12 C07D207/16 A61P37/06 A61K31/34
A61K31/425 A61K31/40 //((C07D405/12,307:00,207:00),
(C07D417/12,307:00,277:00))

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

CHEM ABS Data, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 04247 A (ZHENG ZHONGLI ;ADAMS STEVEN P (US); BIOGEN INC (US); ENSINGER CARO) 5 February 1998 (1998-02-05) Tables 1 to 3; pages 162 to 183 claims 5,10,7 -----	1-20
A	WO 00 39081 A (ABBOTT LAB) 6 July 2000 (2000-07-06) claims 12,13,20-23 -----	1-20

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

° Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

6 June 2002

Date of mailing of the international search report

13/06/2002

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
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Fax: (+31-70) 340-3016

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Goss, I

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 01/44203

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9804247	A 05-02-1998	AU 3738597 A AU 737372 B2 AU 3738697 A BG 103193 A CN 1230110 A CZ 9900232 A3 EE 9900032 A EP 0914605 A1 EP 0917462 A1 JP 2000516596 T NO 990338 A NZ 333904 A PL 331332 A1 SK 8199 A3 TR 9900781 T2 WO 9804913 A1 WO 9804247 A1	20-02-1998 16-08-2001 20-02-1998 30-09-1999 29-09-1999 16-06-1999 16-08-1999 12-05-1999 26-05-1999 12-12-2000 25-03-1999 23-06-2000 05-07-1999 10-04-2000 21-07-1999 05-02-1998 05-02-1998
WO 0039081	A 06-07-2000	US 6110922 A AU 2220300 A BG 105732 A CN 1350520 T CZ 20012412 A3 EP 1140814 A2 NO 20013241 A WO 0039081 A2	29-08-2000 31-07-2000 28-02-2002 22-05-2002 17-04-2002 10-10-2001 28-08-2001 06-07-2000

FURTHER INFORMATION CONTINUED FROM PCT/SA/ 210

Continuation of Box I.2

Claims Nos.: 1,2,3,6,7,9-20 Completely:4,5,8

Present claims 1 to 3 relate to an extremely large number of possible compounds. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a small proportion of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the compounds wherein Cy is nearer defined (namely according to claims 4, 5 and 8 or description page 21, lines 5 to 27).

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/44203

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 7 C07D405/12 C07D417/12 C07D207/16 A61P37/06 A61K31/34
 A61K31/425 A61K31/40 // (C07D405/12, 307:00, 207:00),
 (C07D417/12, 307:00, 277:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 IPC 7 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

CHEM ABS Data, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 04247 A (ZHENG ZHONGLI ;ADAMS STEVEN P (US); BIOGEN INC (US); ENSINGER CARO) 5 February 1998 (1998-02-05) Tables 1 to 3; pages 162 to 183 claims 5,10,7 -----	1-20
A	WO 00 39081 A (ABBOTT LAB) 6 July 2000 (2000-07-06) claims 12,13,20-23 -----	1-20

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- *A* document defining the general state of the art which is not considered to be of particular relevance
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- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

6 June 2002

Date of mailing of the international search report

13/06/2002

Name and mailing address of the ISA

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20. A pharmaceutical composition comprising a compound according to claim 1 with a pharmaceutically acceptable adjuvant, diluent or carrier.
- 10 21. A method of inhibiting binding of a LFA-1 to a protein ligand comprising contacting LFA-1 with a compound of claim 1.
22. A method of treating a disease or condition mediated by LFA-1 in a mammal comprising administering to said mammal an effective amount of a compound according to claim 1.
23. A method according to claim 2, wherein said disease or condition is arthritis, psoriasis, organ transplant rejection, asthma, and inflammatory bowel disease
- 15 24. A method of inhibiting an inflammatory disease or condition in a mammal comprising administering to said mammal an effective amount of a compound according to claim 1.

5 phenyl is optionally substituted with halogen or hydroxyl.

10 13. A compound according to claim 1, wherein L is a divalent hydrocarbon optionally having one or more carbon atoms replaced with N, O, S, SO or SO₂ and optionally being substituted with hydroxyl, halogen oxo or thio; or three carbon atoms of the hydrocarbon are replaced with an amino acid residue.

15 14. A compound according to claim 13, wherein L is -CH=CH-C(O)-NR₆-CH₂-, -CH₂-NR₆-C(O)-, -C(O)-N₆-CH₂-, -CH(OH)-(CH₂)₂-, -(CH₂)₂-CH(OH)-, -(CH₂)₃-, -C(O)-NR₆-CH(R₇)-C(O)-NR₆-, -NR₆-C(O)-CH(R₇)-NR₆-C(O)-, -CH(OH)-CH₂-O- or -CH(OH)-CF₂-CH₂- wherein each R₆ is independently H or alkyl and R₇ is an amino acid side chain.

20 15. A compound according to claim 14, wherein R₁ is H, OH, amino, O-carbocycle or alkoxy optionally substituted with a carbocycle.

25 16. A compound according to claim 15, wherein R₁ is H or C₁₋₄ alkylxy.

30 17. A compound according to claim 1, wherein at least one of R₂ and R₃ is halogen and the other is H or halogen.

35 18. A compound according to claim 17, wherein R₂ and R₃ are both Cl.

19. A compound according to claim 18, wherein R₄ and R₅ are both H.

5 piperidine, piperazine, morpholine, tetrahydrofuran, tetrahydrothiophene, oxazolidine, thiazolidine optionally substituted with hydroxy, oxo, mercapto, thio, alkyl or alkanoyl.

10 6. A compound according to claim 1, wherein Cy is a 3-6 member carbocycle optionally substituted with hydroxyl, mercapto, halogen, oxo, thio, amino, amidine, guanidine, alkyl, alkoxy or acyl.

15 7. A compound according to claim 6, wherein said carbocycle is partially unsaturated.

20 8. A compound according to claim 7, wherein Cy is cyclopropyl, cyclypropenyl, cyclobutyl, cyclbutenyl, cyclopentyl, cyclopentenyl cyclohexyl or cyclohexenyl.

25 9. A compound according to claim 1, wherein X is a C₁₋₅ divalent hydrocarbon optionally having one or more carbon atoms replaced with N, O, S, SO or SO₂ and optionally being substituted with hydroxyl, oxo or thio.

30 10. A compound according to claim 1, wherein X is -CH₂-NR₆-C(O)- wherein the carbonyl -C(O)- portion thereof is covalently bound to Cy and R₆ is H or alkyl.

35 11. A compound according to claim 1, wherein Y is a carbocycle or heterocycle optionally substituted with hydroxyl or halogen.

 12. A compound according to claim 11, wherein Y is furan-2-yl, thiophene-2-yl or phenyl, wherein said

5 R₂₋₅ are independently H, hydroxyl, mercapto, halogen, cyano, amino, amidine, guanidine, nitro or alkoxy; or R₃ and R₄ together form a fused carbocycle or heterocycle optionally substituted with hydroxyl, halogen, oxo, thio, amino, amidine, guanidine or alkoxy;

10 R₆ is H or a hydrocarbon chain optionally substituted with a carbocycle or a heterocycle; and salts, solvates and hydrates thereof;

15 with the proviso that when Y is phenyl, R₂, R₄ and R₅ are H, R₃ is Cl and R₁ is OH then X is other than cyclohexyl.

2. A compound according to claim 1, wherein Cy is a 5- or 6-member non-aromatic heterocycle optionally substituted with hydroxyl, mercapto, thioalkyl halogen, oxo, thio, amino, aminoalkyl, amidine, guanidine, nitro, alkyl, alkoxy or acyl.

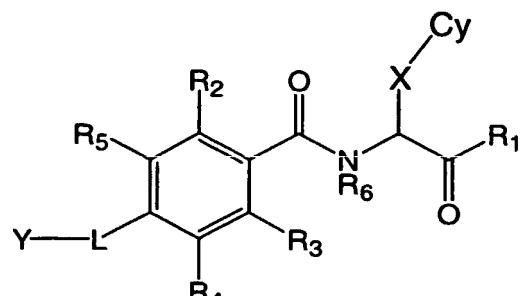
20 3. A compound according to claim 2, wherein said heterocycle comprises one or two heteroatoms and is optionally substituted with hydroxyl, oxo, mercapto, thio, alkyl or alkanoyl.

25 4. A compound according to claim 3, wherein said heterocycle is selected from the group consisting of piperidine, piperazine, morpholine, tetrahydrofuran, tetrahydrothiophene, oxazolidine, cyclopropa-pyrrolidine and thiazolidine optionally substituted with hydroxy, oxo, mercapto, thio, alkyl or alkanoyl.

30 5. A compound according to claim 4, wherein said heterocycle is selected from the group consisting of

5 WE CLAIM:

1. A compound of formula (I)



(I)

wherein

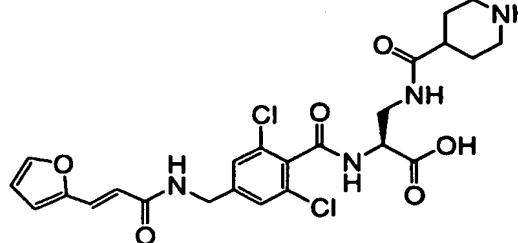
10 Cy is a non-aromatic carbocycle or heterocycle optionally substituted with hydroxyl, mercapto, thioalkyl, halogen, oxo, thio, amino, aminoalkyl, amidine, guanidine, nitro, alkyl, alkoxy or acyl;

15 X is a divalent hydrocarbon chain optionally substituted with hydroxyl, mercapto, halogen, amino, aminoalkyl, nitro, oxo or thio and optionally interrupted with N, O, S, SO or SO₂;

20 Y is a carbocycle or heterocycle optionally substituted with hydroxyl, mercapto, halogen, oxo, thio, thioalkyl, amino, aminoalkyl, carbocycle or heterocycle ring, hydrocarbon, a halo-substituted hydrocarbon, amino, amidine, guanidine, cyano, nitro, alkoxy or acyl;

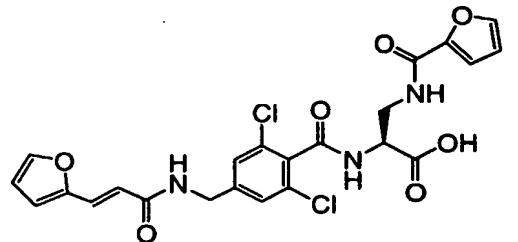
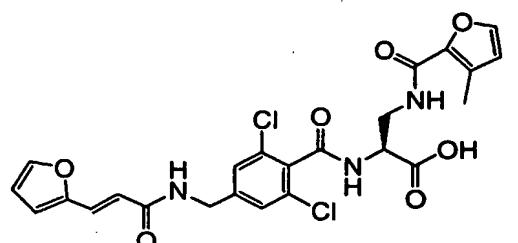
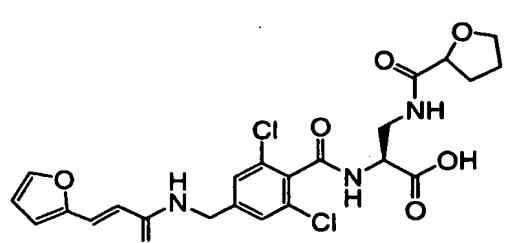
25 L is a bond or a divalent hydrocarbon chain optionally substituted hydroxyl, halogen, oxo or thio and optionally interrupted with N, O, S, SO or SO₂ or an amino acid residue; less than 3 or 5 atoms

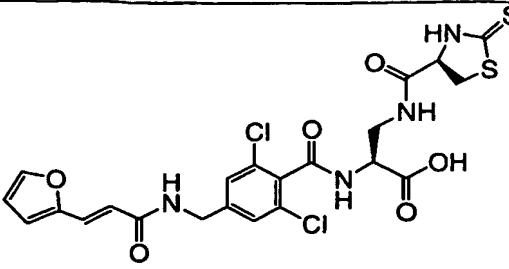
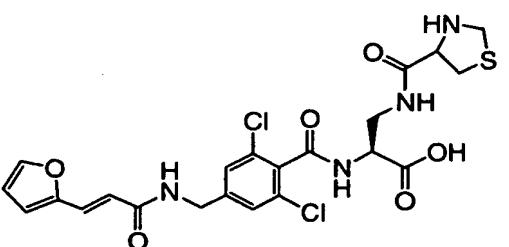
30 R₁ is H, OH, amino, O-carbocycle or alkoxy optionally substituted with amino, a carbocycle or heterocycle;

3	0.011		80.8	
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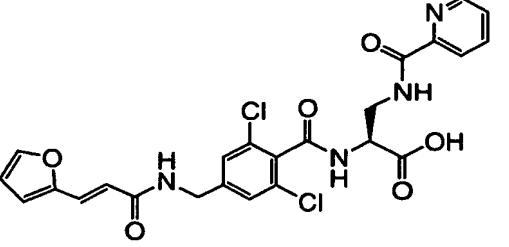
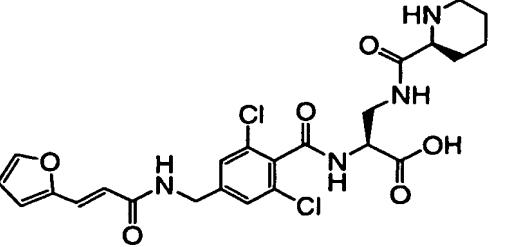
table 4

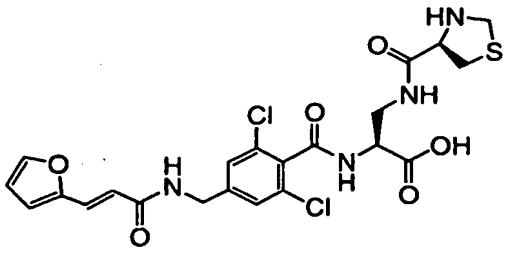
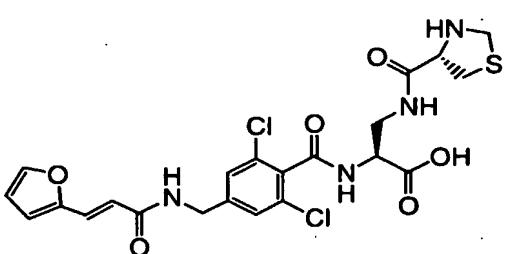
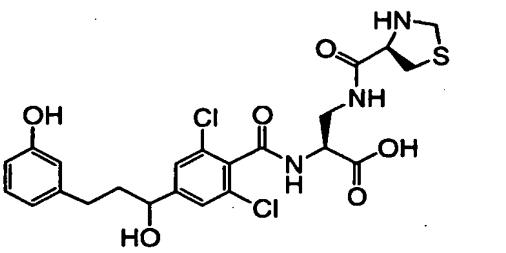
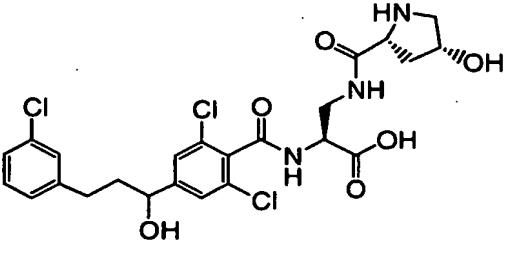
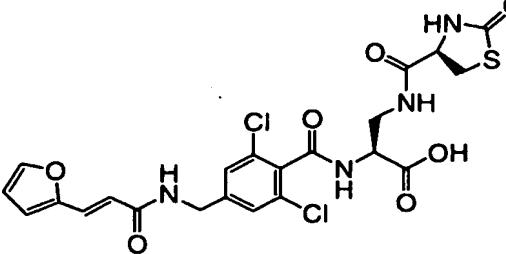
cmpd no.	LFA-1 PLM2 IC ₅₀ (μM)	Mac-1 IC ₅₀ (μM)	% plasma protein binding	structure
ref			99.2	
ref	0.002	1683	99.70	
51	0.005	2362	92.8	

40	0.002	1427	96.93	
42	0.003		91.4	

5

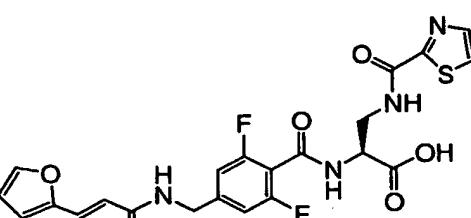
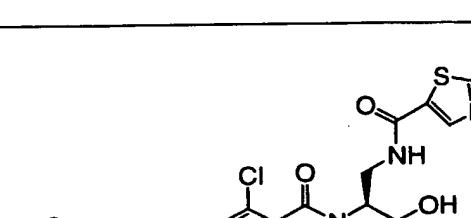
table 3

cmpd no.	LFA-1 PLM2 IC_{50} (μ M)	Mac-1 IC_{50} (μ M)	% plasma protein binding	structure
ref	0.015		99.4	
9	0.002		77.17	

6	0.007	2509	95.43	
15	0.004		92.51	
36	0.002	65	92.84	
37		35.54	93.19	
38	0.012	7609	93.29	

41	0.003	84.83	
44	0.002	82.97	

table 2

cmpd no.	LFA-1 PLM2 IC ₅₀ (μM)	Mac-1 IC ₅₀ (μM)	% plasma protein binding	structure
ref	0.005		98.12	
ref	0.004	161	99.5	

17	0.003		84.61	
10	0.003		65.91	
12	0.002		79.48	
13	0.004		77.58	
14	0.002		72.60	

5 table 1

cmpd no.	LFA-1 PLM2 IC_{50} (μ M)	Mac-1 IC_{50} (μ M)	% plasma protein binding	structure
ref	0.071		98.3	
4	0.004		82.9	
5	0.008		83.1	
35	0.009		51.36	

5 EXAMPLE 12 serum/plasma protein binding

Binding of test compounds was performed according to procedures described in Borga et al (Journal of Pharmacokinetics & Biopharmaceutics, 1997, 25(1):63-77) and Godolphin et al (Therapeutic drug monitoring, 1983, 5:319-23). Duplicate samples of 10 μ l of test compound stock solution (1 μ g/ μ L) was spiked into 1 mL of either buffer or serum/plasma adjusted to pH 7.4 using CO₂ at room temperature. Samples were equilibrated by incubating vials in a water bath with shaker at 37°C for 15 minutes. 10 200 μ l of the buffer spiked sample was saved as prefiltrate. 800 μ l of buffer spiked samples and 1 ml of serum spiked samples were centrifuged at 1500 g, 37°C, for 30 minutes in a Centrifree ultrafiltration device (Amicon Inc.). Pre and post-filtrates were then analyzed by LC/MS-MS and percent binding of test compound to serum/plasma protein was determined from the post and prefiltrates accounting for any non-specific binding determined from the buffer control.

25 Compounds of the invention incorporating a non-aromatic ring at substituent Cy surprisingly exhibit low serum plasma protein binding characteristics which is advantageous for maintaining therapeutically relevant 30 serum levels. As illustrated in tables 1-4, reference compounds (ref) having an aromatic ring at substituent Cy consistently show higher % plasma protein binding compared to the equivalent compound of the invention having a non-aromatic ring.

5 then purified by reverse phase HPLC, verified by
electrospray mass spectrometry and lyophilized to a
powder.

10

EXAMPLE 11 PLM2 Antibody Capture LFA-1:ICAM-1 Assay

A non-function blocking monoclonal antibody against human CD18, PLM-2 (as described by Hildreth, et al., *Molecular Immunology*, Vol. 26, No. 9, pp. 883-895, 1989), is diluted to 5 μ g/ml in PBS and 96-well flat-bottomed plates are coated with 100 μ l/well overnight at 4°C. The plates are blocked with 0.5% BSA in assay buffer (0.02M Hepes, 0.15M NaCl, and 1mM MnCl₂) 1h at room temperature.
Plates are washed with 50mM Tris pH 7.5, 0.1M NaCl, 0.05% Tween 20 and 1mM MnCl₂. Purified full-length recombinant human LFA-1 protein is diluted to 2 μ g/ml in assay buffer and 100 μ l/well is added to plates and incubated 1h at 37°C. Plates are washed 3X. 50 μ l/well inhibitors, appropriately diluted in assay buffer, are added to a 2X final concentration and incubated for 30' at 37°C. 50 μ l/well of purified recombinant human 5 domain ICAM-Ig, diluted to 161ng/ml (for a final concentration of 80ng/ml) in assay buffer, is added and incubated 2h at 37°C. Plates are washed and bound ICAM-Ig is detected with Goat anti-HuIgG(Fc)-HRP for 1h at room temperature. Plates are washed and developed with 100 μ l/well TMB substrate for 5-10' at room temperature. Colorimetric development is stopped with 100 μ l/well 1M H₃PO₄ and read at 450nm on a platereader. Results of the PLM2 assay are shown in tables 1-4 below.

5 To 1 equivalent of this amine was added 1.05 equivalents
of methyl iodide and 2.1 equivalents potassium carbonate
in DMF. The reaction was stirred at room temperature and
followed by TLC (9/1 DCM/MeOH). Upon completion of the
reaction, it was diluted with EtOAc and H₂O. The aqueous
10 layer was partitioned again with EtOAc and the combined
organic layers washed with brine, dried over MgSO₄ and
concentrated *in vacuo*.

15 1 equivalent of this amine, 2 equivalents of Boc-L-
thiazolidine-4-carboxylic acid, 2 equivalents of EDC, 1
equivalent of Hobt and 3 equivalents of DIPEA were
dissolved DMA. The reaction was stirred at room
temperature and monitored by TLC (9/1 DCM/MeOH). Upon
completion, the mixture was concentrated *in vacuo*. The
20 resulting oil was re suspended in Et₂O and washed twice
with 0.1 N H₂SO₄, twice with saturated NaHCO₃, and once
with brine. The organic layer was then dried over MgSO₄,
filtered and concentrated *in vacuo*. The residue was then
purified on silica gel using 5% methanol in DCM as eluent
25 to provide pure methyl ester.

30 1 equivalent of the resultant methyl ester was dissolved
in THF/H₂O (3/1) and 3 equivalents of LiOH•H₂O was added.
The reaction was monitored by TLC (9/1 DCM/MeOH). Upon
completion, the mixture was acidified to pH 2 with 1M HCl
and then concentrated *in vacuo*. The resulting solid was
re suspended in Et₂O and washed twice with 0.1 M HCl and
once with brine. The organic layer was then dried over
MgSO₄, filtered and concentrated *in vacuo*.

35 The residue was dissolved in a solution of TFA in DCM
(1:1). After 20 minutes, the reaction was concentrated *in
vacuo*. The resulting oil was dissolved in toluene and
then re concentrated *in vacuo*. The resulting acid was

5

2.3 equivalents of lithium iodide was added to 1 equivalent of the methyl ester in pyridine, and the mixture heated at reflux for 8 hours. The reaction was concentrated *in vacuo* and the residue was partitioned
10 between EtOAc and 1N HCl. The aqueous layer was extracted three times with EtOAc, and the combined organic layers were washed with 1M NaHCO₃, dried over MgSO₄ and concentrated *in vacuo*. The residue was dissolved in NMM and the solution concentrated *in vacuo*. The residue was
15 taken up in DCM and then washed three times with 1N HCl. The organic layer was dried over MgSO₄ and concentrated *in vacuo* to provide the benzoic acid in high enough purity to be used without further purification.

20 1 equivalent of the acid, 2 equivalents of commercially available β - Boc- diaminopropionic acid methyl ester, 2 equivalents of EDC, 1 equivalent of Hobt and 3 equivalents of DIPEA were dissolved DMA. The reaction was stirred at room temperature and monitored by TLC (9/1
25 DCM/MeOH). Upon completion, the mixture was concentrated *in vacuo*. The resulting oil was re suspended in Et₂O and washed twice with 0.1 N H₂SO₄, twice with saturated NaHCO₃, and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*. The
30 residue was then purified on silica gel using 5% methanol in DCM as eluent to provide pure methyl ester.

The Boc protected amine was dissolved in a solution of TFA in DCM (1:1). After 20 minutes, the reaction was
35 concentrated *in vacuo*. The resulting oil was dissolved in toluene and then re concentrated *in vacuo*.

5 starting material was then degassed while stirring with CO for 10 minutes. 0.15 equivalents palladium(II) acetate and 0.15 equivalents of 1, 3- bis(diphenylphosphino) propane were then added and the mixture was then degassed while stirring with CO for another 10 minutes at which time 10 2.5 equivalents of diisopropyl ethyl amine was added. After properly assembling the bomb, it was charged with 300psi CO gas and heated to 70°C with stirring overnight. The bomb was then cooled and vented. The mixture was transferred to a round bottom flask and 15 concentrated *in vacuo*. The residue was then purified on silica gel using DCM with 1% acetone and 1% TEA as eluent to provide pure methyl ester.

20 The Boc protected amine was dissolved in a solution of TFA in DCM (1:1). After 20 minutes, the reaction was concentrated *in vacuo*. The resulting oil was dissolved in toluene and then reconcentrated *in vacuo*. The TFA salt of the amine was dissolved in Et₂O and washed twice with a 25 10% solution of K₂CO₃ in H₂O and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*.

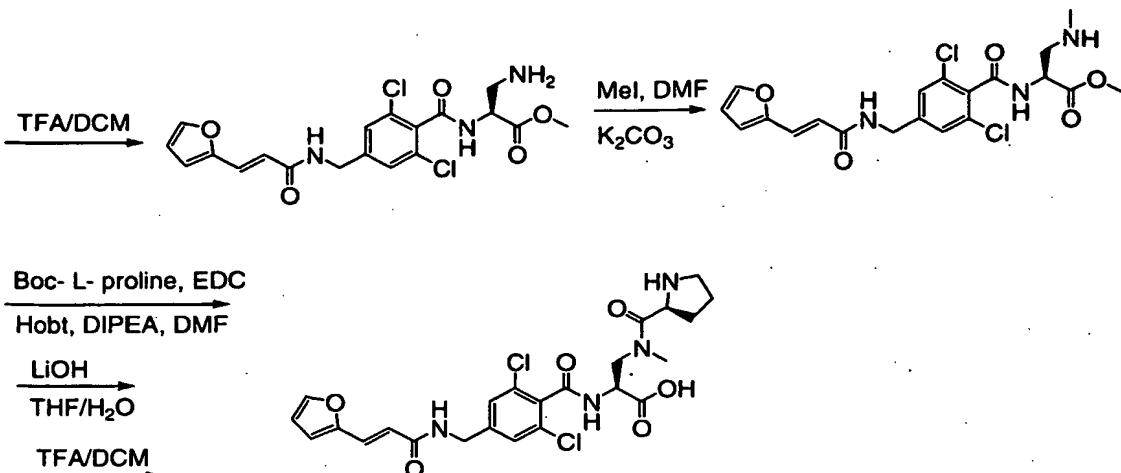
30 1 equivalent of the free based amine, 3 equivalents of furylacrylic acid, 3 equivalents of EDC and 1 equivalent of HObt were dissolved DMA. The reaction was stirred at room temperature and monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was concentrated *in vacuo*. The resulting oil was re suspended in Et₂O and washed twice with 0.1 N H₂SO₄, twice with saturated NaHCO₃, and 35 once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was then purified on silica get using 5% methanol in DCM as eluent to provide pure methyl ester.

5 addition, the mixture was refluxed overnight (> 8 hours). The reaction was cooled to 0°C and the precipitated by-product was removed by filtration. The filtrate was then concentrated *in vacuo*.

10 The crude amine residue was dissolved in a 3:2 THF/H₂O solution. 1.1 equivalents of solid NaHCO₃ and 1.1 equivalents of Boc₂O were added and the mixture was stirred overnight. The reaction was concentrated, and the residue was partitioned between H₂O and Et₂O. The aqueous 15 layer was extracted with Et₂O and the combined organic layers were dried over MgSO₄ and concentrated *in vacuo* to a solid. Recrystallization from hot methanol and H₂O provided pure product.

20 1 equivalent of the Boc protected amine and 1.5 equivalents of 2, 6-lutidine was dissolved, with mild heating if necessary, in DCM in a round bottom flask. Once the starting material has completely dissolved, the mixture was cooled to -78°C under N₂ with a dry ice 25 ethanol bath. Once cool, 2.5 equivalents of triflic anhydride was added and the reaction was allowed to slowly come to room temperature with stirring. The reaction was monitored by TLC and was generally done in 4 hours. Upon completion, the reaction was concentrated *in 30 vacuo* and the residue partitioned between EtOAc and H₂O. The organic layer was washed twice with 0.1N H₂SO₄, twice with saturated NaHCO₃, once with brine, dried over MgSO₄ and concentrated *in vacuo*. The residue was then purified 35 on silica gel using DCM as eluent to provide pure triflate.

1 equivalent of triflate was dissolved in DMF and MeOH in the glass insert of a high pressure Parr bomb. The



A round bottom flask was equipped with an efficient overhead stirrer and charged with concentrated H₂SO₄ (2.7 x volume of H₂O) and H₂O and cooled to ~-5°C with an ethanol/ice bath. Once cool, 1 equivalent 2.6 dichloro phenol and 1 equivalent of N-(hydroxymethyl)phthalimide were added with vigorous stirring. The reaction was kept cool for 4 hours and then allowed to warm to room temperature overnight with constant stirring. The reaction generally proceeds to a point where there was just a solid in the round bottom flask. At this point EtOAc and H₂O were added and stirred into the solid. Large chunks were broken up and then the precipitate was filtered and washed with more EtOAc and H₂O. The product was then used without further purification after drying overnight under vacuum.

1 equivalent of the dry product and methanol (22.5ml x #g of starting material) was added to a round bottom flask equipped with a H₂O condenser and stirring bar. 1.2 equivalents of hydrazine mono hydrate was added and the mixture refluxed for 4 hours. After cooling to room temperature, concentrated HCl (4.5ml x #g of starting material) was carefully added. Upon completion of the

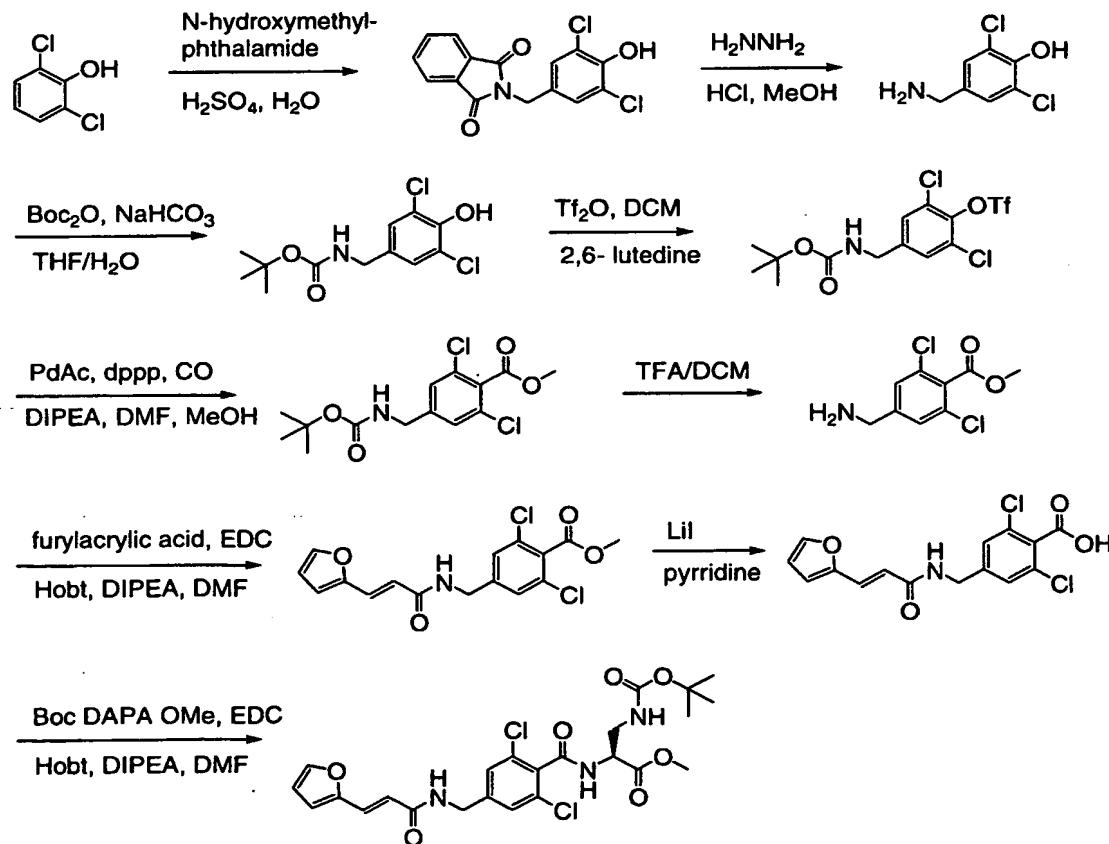
5 The reaction was monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was acidified to pH 2 with 1M HCl and then concentrated *in vacuo*. The resulting solid was re suspended in Et₂O and washed twice with 0.1 M HCl and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*. The Boc, silyl residue was dissolved in a solution of TFA in DCM (1:1). After 20 minutes, the reaction was concentrated *in vacuo*. The resulting oil was dissolved in toluene and then reconcentrated *in vacuo*. The resulting acid was then purified by reverse phase HPLC, verified by electrospray mass spectrometry and lyophilized to a powder.

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EXAMPLE 10 Synthesis of compound 35

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5 dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was then purified on silica gel using 5% methanol in DCM as eluent to provide pure methyl ester.

10 1 equivalent of commercially available D-hydroxy proline was dissolved in a 3:2 THF/H₂O solution. 1.1 equivalents of solid NaHCO₃ and 1.1 equivalents of Boc₂O were added and the mixture was stirred overnight. The reaction was concentrated to remove the THF, and the resulting aqueous layer was partitioned with hexanes. The aqueous layer was then acidified to pH 2 with 1N HCl and then partitioned twice with EtOAc. The combined organic layers were dried over MgSO₄ and concentrated *in vacuo*. The resulting N-Boc-D-hydroxy proline was used without further purification.

20 The Boc protected amine was dissolved in a solution of TFA in DCM (1:1). After 20 minutes, the reaction was concentrated *in vacuo*. The resulting oil was dissolved in toluene and then reconcentrated *in vacuo*. 1 equivalent of this amine, 2 equivalents of Boc-D-hydroxy proline, 2 equivalents of EDC, 1 equivalent of HObt and 3 equivalents of DIPEA were dissolved DMA. The reaction was stirred at room temperature and monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was concentrated *in vacuo*. The resulting oil was re suspended in Et₂O and washed twice with 0.1 N H₂SO₄, twice with saturated NaHCO₃, and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was then purified on silica gel using 5% methanol in DCM as eluent to provide pure methyl ester.

35 1 equivalent of the resultant methyl ester was dissolved in THF/H₂O (3/1) and 3 equivalents of LiOH•H₂O was added.

5 1 equivalent of the aryl alkyne was dissolved in MeOH and
the solution was degassed by passing N₂ through a pipette
and into the solution for 10 minutes. The 5% Rh/Al₂O₃ was
added, one balloon-full of hydrogen was passed through
the solution, and the reaction was stirred under an
10 atmosphere of H₂ (using a balloon) for 7 hours, after
which the reaction was filtered through a pad of celite
and concentrated *in vacuo*. The residue was purified by
silica gel flash chromatography (gradient elution, using
Et₂O to EtOAc) to provide the pure product.

15 2.3 equivalents of lithium iodide was added to 1
equivalent of the methyl ester in pyridine, and the
mixture heated at reflux for 8 hours. The reaction was
concentrated *in vacuo* and the residue was partitioned
20 between EtOAc and 1N HCl. The aqueous layer was extracted
three times with EtOAc, and the combined organic layers
were washed with 1M NaHCO₃, dried over MgSO₄ and
concentrated *in vacuo*. The residue was dissolved in NMM
and the solution concentrated *in vacuo*. The residue was
25 taken up in DCM and then washed three times with 1N HCl.
The organic layer was dried over MgSO₄ and concentrated *in
vacuo* to provide the benzoic acid in high enough purity
to be used without further purification.

30 1 equivalent of the acid, 2 equivalents of commercially
available β - Boc- diaminopropionic acid methyl ester, 2
equivalents of EDC, 1 equivalent of Hobt and 3
equivalents of DIPEA were dissolved DMA. The reaction was
stirred at room temperature and monitored by TLC (9/1
35 DCM/MeOH). Upon completion, the mixture was concentrated
in vacuo. The resulting oil was re suspended in Et₂O and
washed twice with 0.1 N H₂SO₄, twice with saturated
NaHCO₃, and once with brine. The organic layer was then

5 washed with saturated NaHCO₃ and brine, then dried over MgSO₄ and concentrated *in vacuo* to an oil. Toluene was added to the oil and the solution concentrated *in vacuo* again. This was repeated to obtain a crystalline solid. The solid was dissolved in methanol and concentrated HCl 10 and heated at reflux for 12 hours. The reaction was concentrated *in vacuo* and the residue was purified by silica gel flash chromatography (9:1 hexane/Et₂O) to provide the pure aldehyde.

15 A solution of 1 equivalent of the aldehyde in THF was cooled to -78°C and 1.1 equivalents of 0.5M ethynylmagnesium bromide/THF was added. After stirring the reaction at room temperature for 3 hours, it was diluted with Et₂O and washed twice with 10% citric acid. 20 The combined aqueous layers were back-extracted once with Et₂O. The combined organic layers were washed twice with saturated aqueous NaHCO₃, dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by silica gel flash chromatography (4:1 to 3:2 hexane/Et₂O) to 25 provide the pure alkyne.

1 1 equivalent of 1-chloro-3-iodobenzene was dissolved in EtOAc and the solution was degassed by passing N₂ through a pipette and into the solution for 10 minutes. 1.25 30 equivalents of the alkyne was added, followed by 0.02 equivalents of dichlorobis(triphenylphosphine)palladium-(II), 0.04 equivalents of CuI and 5 equivalents TEA. The reaction was stirred for 14 hours, diluted with EtOAc, washed twice with 5% Na₂•EDTA, brine and then dried over 35 MgSO₄ and concentrated *in vacuo*. The residue was purified by silica gel flash chromatography (gradient elution, using Et₂O to EtOAc) to provide the pure aryl alkyne.

5 adding 1.25 equivalents of triflic anhydride the stirring reaction was allowed to warm to room temperature overnight. The reaction was then concentrated, and the residue was partitioned between Et₂O and H₂O. The aqueous layer was extracted with Et₂O and the combined organic
10 layers were dried over MgSO₄ and concentrated in vacuo. The residue was purified by silica gel flash chromatography (9:1 hexane/Et₂O) to provide the pure triflate.

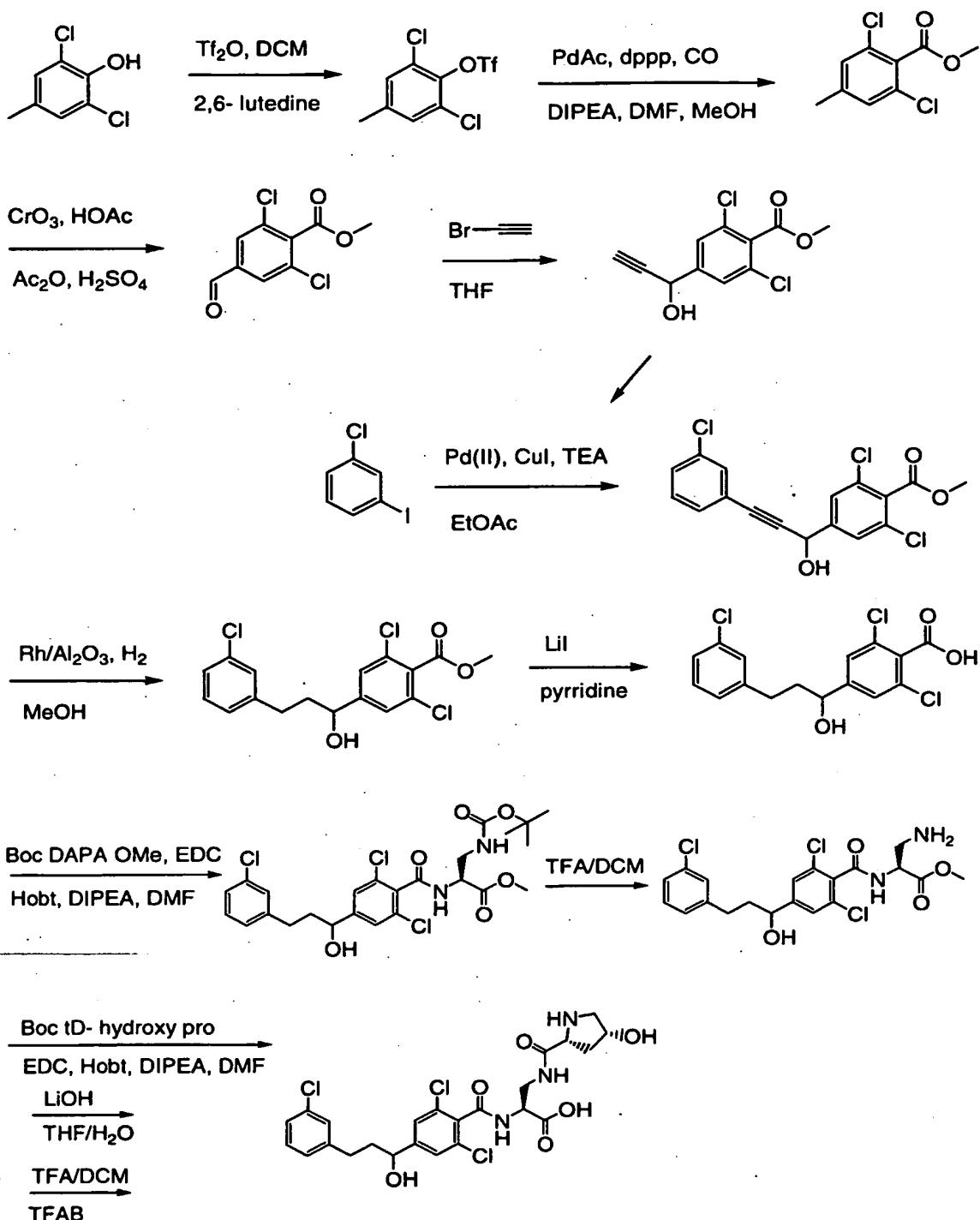
15 To a stirring solution of 1 equivalent of the triflate in a 2/1 mixture of DMF/MeOH was added 0.15 equivalents of 1, 3-bis(diphenylphosphino)-propane and 2.5 equivalents of TEA. Carbon monoxide gas was bubbled through this solution for 15 minutes, then 0.15 equivalents of
20 Pd(OAc)₂ was added and the reaction was stirred at 70°C for 5-7 hours under an atmosphere of CO (using a balloon filled with CO). The reaction was then concentrated in vacuo, and the residue was partitioned between Et₂O and H₂O. The aqueous layer was extracted twice with Et₂O and
25 the combined organic layers were dried over MgSO₄, filtered through a plug of silica gel and concentrated in vacuo. The residue was purified by silica gel flash chromatography (9:1:0.02 hexane/DCM/Et₂O) to provide the pure tolyl methyl ester.

30 1 equivalent of the tolyl methyl ester was dissolved in acetic anhydride and HOAc, then cooled in an ice-salt bath (-5°C) before concentrated H₂SO₄ was added. A solution of CrO₃ (2.6 equivalents) in acetic anhydride and
35 HOAc was added drop wise and the reaction was stirred for 3.5 hours at -5°C. The reaction was poured into ice H₂O and stirred for 30 min. The mixture was extracted three times with ethyl ether. The combined organic layers were

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EXAMPLE 9

Synthesis of compounds 37



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1 equivalent of 2, 6-Dichloro-4-methyl phenol was dissolved in DCM containing 2.6 equivalents of 2, 6-lutidine and the mixture was cooled to -78°C. After

5 The Boc protected amine was dissolved in a solution of TFA in DCM (1:1). After 20 minutes, the reaction was concentrated *in vacuo*. The resulting oil was dissolved in toluene and then reconcentrated *in vacuo*. 1 equivalent of this amine, 2 equivalents of Boc-L-thiazolidine-4-carboxylic acid, 2 equivalents of EDC, 1 equivalent of Hobt and 3 equivalents of DIPEA were dissolved DMA. The reaction was stirred at room temperature and monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was concentrated *in vacuo*. The resulting oil was re suspended in Et₂O and washed twice with 0.1 N H₂SO₄, twice with saturated NaHCO₃, and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was then purified on silica gel using 5% methanol in DCM as eluent to provide pure methyl ester.

1 equivalent of the resultant methyl ester was dissolved in THF/H₂O (3/1) and 3 equivalents of LiOH•H₂O was added. The reaction was monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was acidified to pH 2 with 1M HCl and then concentrated *in vacuo*. The resulting solid was re suspended in Et₂O and washed twice with 0.1 M HCl and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*.

30 The Boc, silyl residue was dissolved in a solution of TFA in DCM (1:1) with 3 equivalents of TBAF. After 20 minutes, the reaction was concentrated *in vacuo*. The resulting oil was dissolved in toluene and then reconcentrated *in vacuo*. The resulting acid was then purified by reverse phase HPLC, verified by electrospray mass spectrometry and lyophilized to a powder.

5 atmosphere of H₂ (using a balloon) for 7 hours, after which the reaction was filtered through a pad of celite and concentrated in vacuo. The residue was purified by silica gel flash chromatography (gradient elution, using Et₂O to EtOAc) to provide the pure product.

10 2.3 equivalents of lithium iodide was added to 1 equivalent of the methyl ester in pyridine, and the mixture heated at reflux for 8 hours. The reaction was concentrated in vacuo and the residue was partitioned
15 between EtOAc and 1N HCl. The aqueous layer was extracted three times with EtOAc, and the combined organic layers were washed with 1M NaHCO₃, dried over MgSO₄ and concentrated in vacuo. The residue was dissolved in NMM and the solution concentrated in vacuo. The residue was taken up in DCM and then washed three times with 1N HCl. The organic layer was dried over MgSO₄ and concentrated in vacuo to provide the benzoic acid in high enough purity to be used without further purification.

25 1 equivalent of the acid, 2 equivalents of commercially available β - Boc- diaminopropionic acid methyl ester, 2 equivalents of EDC, 1 equivalent of Hobt and 3 equivalents of DIPEA were dissolved DMA. The reaction was stirred at room temperature and monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was concentrated in vacuo. The resulting oil was re suspended in Et₂O and washed twice with 0.1 N H₂SO₄, twice with saturated NaHCO₃, and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated in vacuo. The residue was then purified on silica gel using 5% methanol in DCM as eluent to provide pure methyl ester.

5 Et₂O. The combined organic layers were washed twice with saturated aqueous NaHCO₃, dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by silica gel flash chromatography (4:1 to 3:2 hexane/Et₂O) to provide the pure alkyne.

10

1 equivalent of 3-Iodophenol, 2.2 equivalents of t-butyldimethyl silyl chloride and 3 equivalents of imidazole were dissolved in DMF and stirred at room temperature. The reaction was monitored by TLC (9/1 DCM/MeOH). Upon reaction completion, the mixture was concentrated *in vacuo*. The resulting oil was re suspended in Et₂O and washed twice with saturated NaHCO₃, and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*. The product was then used with out further purification.

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1 equivalent of the silyl iodide was dissolved in EtOAc and the solution was degassed by passing N₂ through a pipette and into the solution for 10 minutes. 1.25 equivalents of the alkyne was added, followed by 0.02 equivalents of dichlorobis(triphenylphosphine)-palladium(II), 0.04 equivalents of CuI and 5 equivalents TEA. The reaction was stirred for 14 hours, diluted with EtOAc, washed twice with 5% Na₂•EDTA, brine and then dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by silica gel flash chromatography (gradient elution, using Et₂O to EtOAc) to provide the pure aryl alkyne.

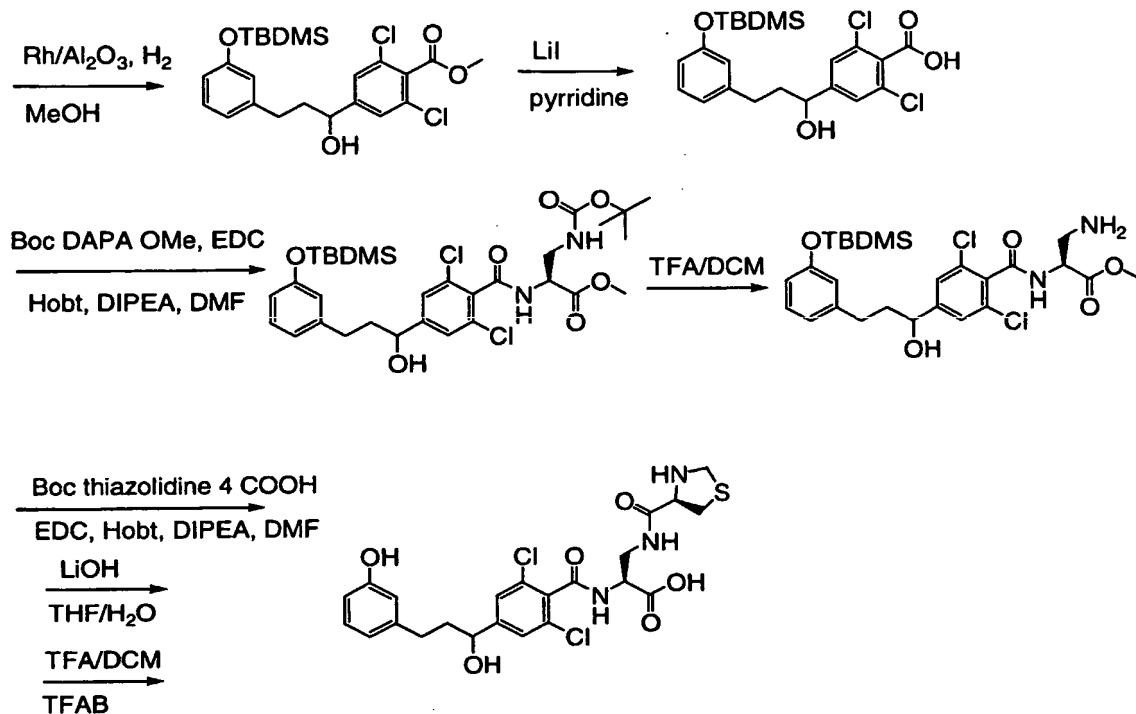
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1 equivalent of the aryl alkyne was dissolved in MeOH and the solution was degassed by passing N₂ through a pipette and into the solution for 10 minutes. The 5% Rh/Al₂O₃ was added, one balloon-full of hydrogen was passed through the solution, and the reaction was stirred under an

5 for 5-7 hours under an atmosphere of CO (using a balloon filled with CO). The reaction was then concentrated *in vacuo*, and the residue was partitioned between Et₂O and H₂O. The aqueous layer was extracted twice with Et₂O and the combined organic layers were dried over MgSO₄,
10 filtered through a plug of silica gel and concentrated *in vacuo*. The residue was purified by silica gel flash chromatography (9:1:0.02 hexane/DCM/Et₂O) to provide the pure tolyl methyl ester.

15 1 equivalent of the tolyl methyl ester was dissolved in acetic anhydride and HOAc, then cooled in an ice-salt bath (-5°C) before concentrated H₂SO₄ was added. A solution of CrO₃ (2.6 equivalents) in acetic anhydride and HOAc was added drop wise and the reaction was stirred for
20 3.5 hours at -5°C. The reaction was poured into ice H₂O and stirred for 30 min. The mixture was extracted three times with ethyl ether. The combined organic layers were washed with saturated NaHCO₃ and brine, then dried over MgSO₄ and concentrated *in vacuo* to an oil. Toluene was
25 added to the oil and the solution concentrated *in vacuo* again. This was repeated to obtain a crystalline solid. The solid was dissolved in methanol and concentrated HCl and heated at reflux for 12 hours. The reaction was concentrated *in vacuo* and the residue was purified by
30 silica gel flash chromatography (9:1 hexane/Et₂O) to provide the pure aldehyde.

A solution of 1 equivalent of the aldehyde in THF was cooled to -78°C and 1.1 equivalents of 0.5M ethynylmagnesium bromide/THF was added. After stirring the reaction at room temperature for 3 hours, it was diluted with Et₂O and washed twice with 10% citric acid. The combined aqueous layers were back-extracted once with



10 1 equivalent of 2, 6-Dichloro-4-methyl phenol was dissolved in DCM containing 2.6 equivalents of 2, 6-lutidine and the mixture was cooled to -78°C. After adding 1.25 equivalents of triflic anhydride the stirring reaction was allowed to warm to room temperature overnight. The reaction was then concentrated, and the residue was partitioned between Et₂O and H₂O. The aqueous layer was extracted with Et₂O and the combined organic layers were dried over MgSO₄ and concentrated in vacuo.

15 The residue was purified by silica gel flash chromatography (9:1 hexane/Et₂O) to provide the pure triflate.

20

To a stirring solution of 1 equivalent of the triflate in a 2/1 mixture of DMF/MeOH was added 0.15 equivalents of 1, 3-bis(diphenylphosphino)-propane and 2.5 equivalents of TEA. Carbon monoxide gas was bubbled through this solution for 15 minutes, then 0.15 equivalents of Pd(OAc)₂ was added and the reaction was stirred at 70°C

5 in Et₂O and washed twice with 0.1 N H₂SO₄, twice with saturated NaHCO₃, and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated in vacuo. The residue was then purified on silica gel using 5% methanol in DCM as eluent to provide pure product.

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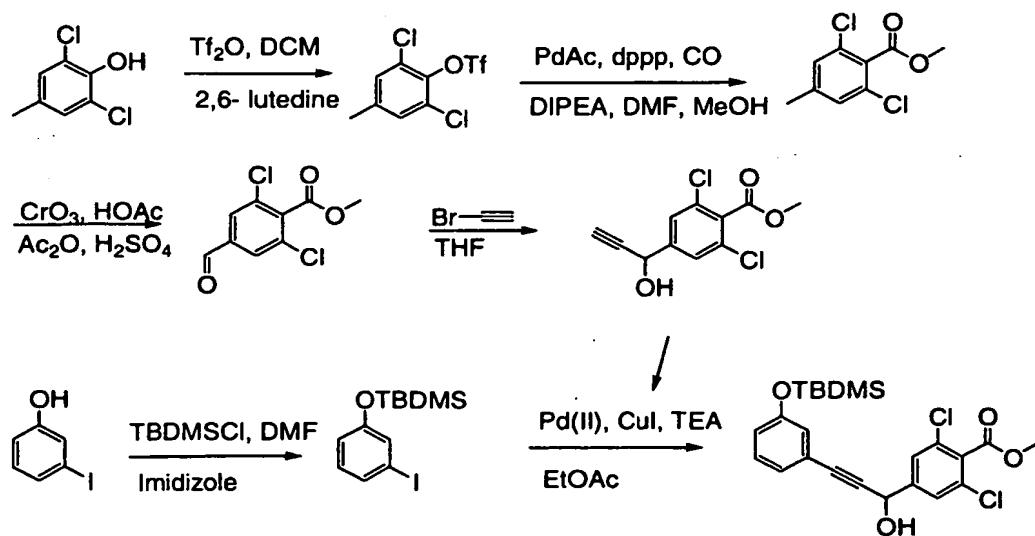
1 equivalent of the resultant methyl ester was dissolved in THF/H₂O (3/1) and 3 equivalents of LiOH·H₂O were added. The reaction was monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was acidified to pH 2 with 1M HCl and then concentrated in vacuo. The resulting solid was re suspended in Et₂O and washed twice with 0.1 M HCl and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated in vacuo. The resulting acid was then purified by reverse phase HPLC, verified by electrospray mass spectrometry and lyophilized to a powder.

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EXAMPLE 8 Synthesis of compounds 36

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5 then acidified to pH 2 with 1N HCl and then partitioned twice with EtOAc. The combined organic layers were dried over MgSO₄ and concentrated in vacuo. The resulting Boc protected isonipecotic acid was used without further purification.

10 The Boc methyl ester was dissolved in a solution of TFA in DCM (1:1). After 20 minutes, the reaction was concentrated in vacuo. The resulting oil was dissolved in toluene and then re concentrated in vacuo. 1 equivalent of this amine, 2 equivalents of resulting Boc protected isonipecotic acid, 2 equivalents of EDC, 1 equivalent of HObt and 3 equivalents of DIPEA were dissolved DMA. The reaction was stirred at room temperature and monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was concentrated in vacuo. The resulting oil was re suspended in Et₂O and washed twice with 0.1 N H₂SO₄, twice with saturated NaHCO₃, and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated in vacuo. The residue was then purified on silica gel using 25 5% methanol in DCM as eluent to provide pure product.

30 This Boc protected product was dissolved in a solution of TFA in DCM (1:1). After 20 minutes, the reaction was concentrated in vacuo. The resulting oil was dissolved in toluene and then concentrated in vacuo twice to provide pure amine. 1 equivalent of this amine, 2 equivalents of the appropriate commercially available acid (example 32; propionic acid; example 33, butyric acid; example 34, acetic acid), 2 equivalents of EDC, 1 equivalent of HObt 35 and 3 equivalents of DIPEA were dissolved DMA. The reaction was stirred at room temperature and monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was concentrated in vacuo. The resulting oil was re suspended

5 concentrated in vacuo. The resulting oil was dissolved in toluene and then concentrated in vacuo twice.

The resulting compound was dissolved in DCM and cooled to -5°C in an ice/acetone bath under nitrogen. 2 equivalents 10 of BBr₃ were added drop wise as a solution in DCM over 30 minutes. The reaction was warmed to room temperature and stirred until complete by TLC (DCM/2% HOAc/2% MeOH). The 15 solution was poured onto ice, and the ice was allowed to melt. The mixture was then partitioned twice with EtOAc and the combined organic layers were dried over MgSO₄. The filtrate was then passed over a plug of silica gel and concentrated in vacuo to afford pure benzoic acid.

20 1 equivalent of the benzoic acid, 2 equivalents of commercially available \square - Boc- diaminopropionic acid methyl ester, 2 equivalents of EDC, 1 equivalent of HObt and 3 equivalents of DIPEA were dissolved DMA. The 25 reaction was stirred at room temperature and monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was concentrated in vacuo. The resulting oil was re suspended in Et₂O and washed twice with 0.1 N H₂SO₄, twice with saturated NaHCO₃, and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated in 30 vacuo. The residue was then purified on silica gel using 5% methanol in DCM as eluent to provide pure Boc methyl ester.

35 1 equivalent of commercially available isonipecotic acid was dissolved in a 3:2 THF/H₂O solution. 1.1 equivalents of solid NaHCO₃ and 1.1 equivalents of Boc₂O were added and the mixture was stirred overnight. The reaction was concentrated to remove the THF, and the resulting aqueous layer was partitioned with hexanes. The aqueous layer was

5 with concentrated HCl and then concentrated in vacuo to
remove the THF. The resulting aqueous layer was washed
twice with Et₂O and the combined organic layers were
washed once with brine. The organic layer was then dried
over MgSO₄, filtered and concentrated in vacuo. The
10 benzoic acid t-butyl ester was used without further
purification.

15 1 equivalent of 3-methoxybenzonitrile was placed in a
Parr bottle with EtOH, 0.02 equivalents of HCl and 10%
(w/w) of 10% Pd on carbon. The vessel was placed in the
Parr shaker, charged with 50psi H₂, and shaken for 12
hours. The reaction filtered through a pad of celite and
diluted 1:10 with Et₂O. Upon standing over night, fine
white needles form. The product was filtered, washed with
20 Et₂O and dried in vacuo. The resulting amine hydrochloride
salt was then used with out further purification.

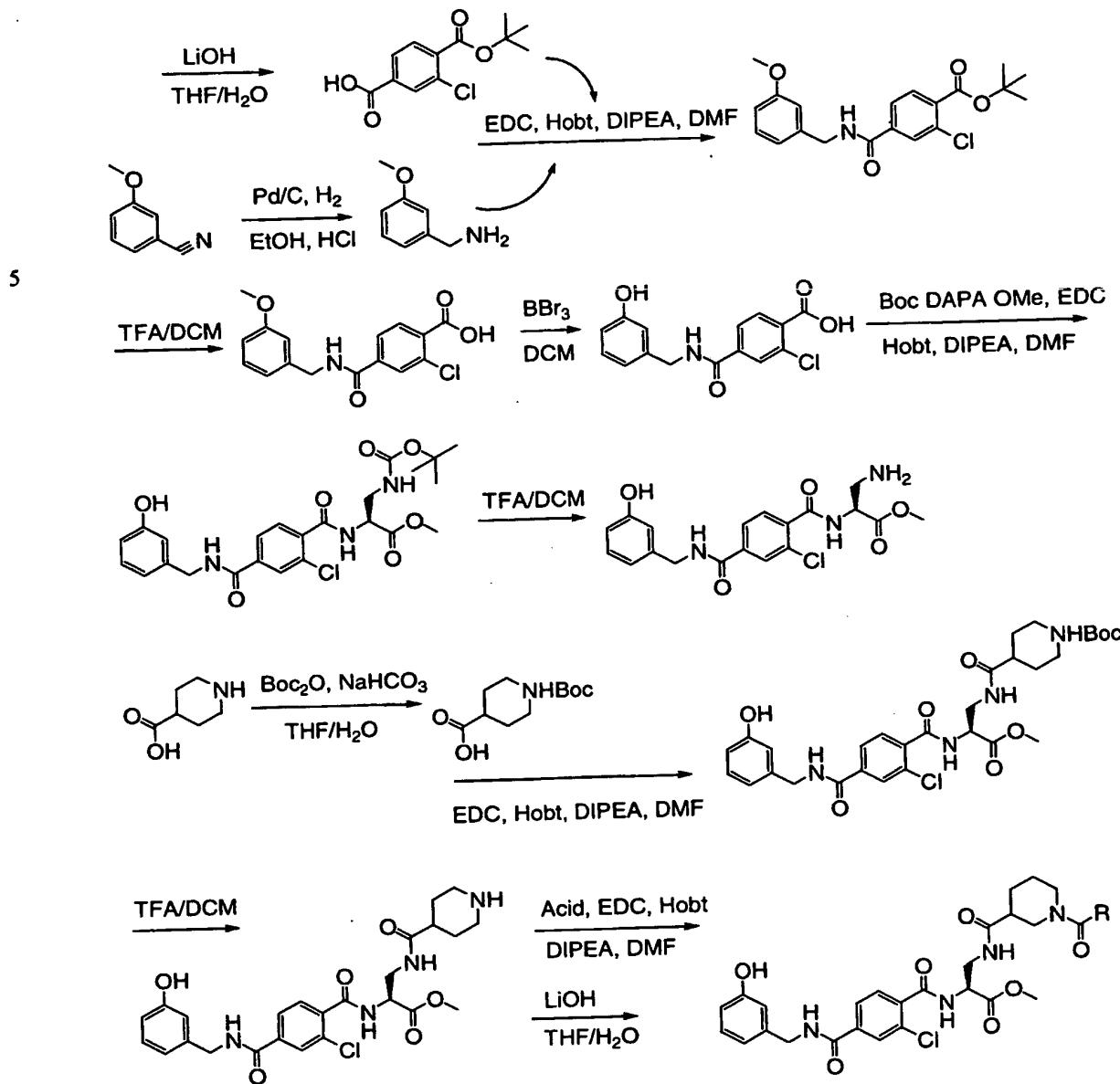
25 3 equivalents of the benzoic acid t-butyl ester was
coupled to 1 equivalent of the amine hydrochloride salt
using 3 equivalents EDC, 1 equivalent of Hobt and 3
equivalents of DIPEA in DMA. The reaction was monitored
by TLC (9/1 DCM/MeOH). Upon completion, the mixture was
concentrated in vacuo. The resulting oil was re suspended
in Et₂O and washed twice with 0.1 N H₂SO₄, twice with
30 saturated NaHCO₃, and once with brine. The organic layer
was then dried over MgSO₄, filtered and concentrated in
vacuo. The product was then purified on silica gel using
5% methanol in DCM as eluent to provide pure t-butyl
ester.

35 The t-butyl ester was dissolved in a solution of TFA in
DCM (1:1). After 20 minutes, the reaction was

5 of saturated NaHCO₃ until the pH remained above 8. This
solution was partitioned one time with and equal volume
of DCM to remove unreacted diester. The basic solution
was acidified at 0°C. with concentrated HCl to pH = 1-
10 1.5, and precipitate was extracted twice with equal
volumes of EtOAc. The organics were partitioned once
with brine and dried over MgSO₄, filtered and concentrated
in vacuo. Product was 7:1 of the correct regioisomer by
HPLC.

15 The monoester was dissolved in DCM and transferred to a
pre-weighed Parr flask containing a stirring bar. The
flask was cooled to -5°C with a dry ice/alcohol bath
under nitrogen. Once cool, ~30 equivalents of isobutylene
was pumped into solution with stirring. 2.1 equivalents
20 of concentrated sulfuric acid was added and the flask was
sealed with a wired rubber stopper and allowed to warm to
room temperature with stirring. The solution was stirred
until clarification (1-2 days). Once the solution was
clear, it was cooled to 0°C in an ice bath. The stopper
25 was removed and the excess isobutylene was blown off with
nitrogen bubbling. Saturated NaHCO₃ was added to
neutralize the acid and the mixture was concentrated in
vacuo until no DCM remained. The solution was then
partitioned into EtOAc. The organics were partitioned
30 twice with dilute HCl, twice with saturated NaHCO₃, once
with brine, dried over MgSO₄, filtered and concentrated in
vacuo. The resulting product was used with no further
purification.

35 1 equivalent of the methyl ester was dissolved in THF/H₂O
(3/1) and 3 equivalents of LiOH·H₂O was added. The
reaction was monitored by TLC (9/1 DCM/MeOH). Upon
completion, the mixture was acidified carefully to pH 2

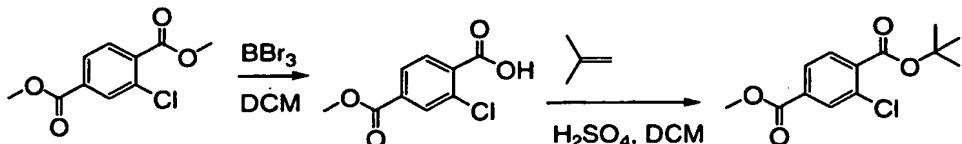


1 equivalent of dimethyl 2- chloroterephthalic acid was dissolved in DCM and cooled to -5°C in an ice/acetone bath under nitrogen. 1 equivalent of BBr₃ was added drop wise as a solution in DCM over 30 minutes. The reaction was warmed to room temperature and stirred until complete by TLC (DCM/2% HOAc/2% MeOH). The solution was poured onto ice, and the ice was allowed to melt. The mixture was then partitioned with EtOAc and concentrated in vacuo. This product was dissolved in H₂O with the addition

5 propionic acid; example 30, acetic acid), 2 equivalents
of EDC, 1 equivalent of HObt and 3 equivalents of DIPEA
were dissolved DMA. The reaction was stirred at room
temperature and monitored by TLC (9/1 DCM/MeOH). Upon
completion, the mixture was concentrated *in vacuo*. The
10 resulting oil was re suspended in Et₂O and washed twice
with 0.1 N H₂SO₄, twice with saturated NaHCO₃, and once
with brine. The organic layer was then dried over MgSO₄,
filtered and concentrated *in vacuo*. The residue was then
purified on silica gel using 5% methanol in DCM as eluent
15 to provide pure product.

1 equivalent of the resultant methyl ester was dissolved
in THF/H₂O (3/1) and 3 equivalents of LiOH·H₂O was added.
The reaction was monitored by TLC (9/1 DCM/MeOH). Upon
20 completion, the mixture was acidified to pH 2 with 1M HCl
and then concentrated *in vacuo*. The resulting solid was
re suspended in Et₂O and washed twice with 0.1 M HCl and
once with brine. The organic layer was then dried over
MgSO₄, filtered and concentrated *in vacuo*. The resulting
25 acid was then purified by reverse phase HPLC, verified by
electrospray mass spectrometry and lyophilized to a
powder.

30 EXAMPLE 7 Synthesis of compounds 32-34



WO 02/059114

5 1 equivalent of commercially available nipecotic acid was dissolved in a 3:2 THF/H₂O solution. 1.1 equivalents of solid NaHCO₃ and 1.1 equivalents of Boc₂O were added and the mixture was stirred overnight. The reaction was concentrated to remove the THF, and the resulting aqueous layer was partitioned with hexanes. The aqueous layer was
10 then acidified to pH 2 with 1N HCl and then partitioned twice with EtOAc. The combined organic layers were dried over MgSO₄ and concentrated in vacuo. The resulting Boc protected nipecotic acid was used without further purification.
15

The Boc methyl ester was dissolved in a solution of TFA in DCM (1:1). After 20 minutes, the reaction was concentrated in vacuo. The resulting oil was dissolved in toluene and then re concentrated in vacuo. 1 equivalent of this amine, 2 equivalents of resulting Boc protected nipecotic acid, 2 equivalents of EDC, 1 equivalent of HObt and 3 equivalents of DIPEA were dissolved DMA. The reaction was stirred at room temperature and monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was concentrated in vacuo. The resulting oil was re suspended in Et₂O and washed twice with 0.1 N H₂SO₄, twice with saturated NaHCO₃, and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated in vacuo. The residue was then purified on silica gel using 5% methanol in DCM as eluent to provide pure product.
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This Boc protected product was dissolved in a solution of TFA in DCM (1:1). After 20 minutes, the reaction was concentrated in vacuo. The resulting oil was dissolved in toluene and then concentrated in vacuo twice to provide pure amine. 1 equivalent of this amine, 2 equivalents of the appropriate commercially available acid (example 29;

5 vacuo. The product was then purified on silica gel using
5% methanol in DCM as eluent to provide pure t-butyl
ester.

10 The t-butyl ester was dissolved in a solution of TFA in
DCM (1:1). After 20 minutes, the reaction was
concentrated *in vacuo*. The resulting oil was dissolved in
toluene and then concentrated *in vacuo* twice.

15 The resulting compound was dissolved in DCM and cooled to
-5°C in an ice/acetone bath under nitrogen. 2 equivalents
of BBr₃ were added drop wise as a solution in DCM over 30
minutes. The reaction was warmed to room temperature and
stirred until complete by TLC (DCM/2% HOAc/2% MeOH). The
solution was poured onto ice, and the ice was allowed to
20 melt. The mixture was then partitioned twice with EtOAc
and the combined organic layers were dried over MgSO₄. The
filtrate was then passed over a plug of silica gel and
concentrated *in vacuo* to afford pure benzoic acid.

25 1 equivalent of the benzoic acid, 2 equivalents of
commercially available \square - Boc- diaminopropionic acid
methyl ester, 2 equivalents of EDC, 1 equivalent of HObt
and 3 equivalents of DIPEA were dissolved DMA. The
reaction was stirred at room temperature and monitored by
30 TLC (9/1 DCM/MeOH). Upon completion, the mixture was
concentrated *in vacuo*. The resulting oil was re suspended
in Et₂O and washed twice with 0.1 N H₂SO₄, twice with
saturated NaHCO₃, and once with brine. The organic layer
was then dried over MgSO₄, filtered and concentrated *in*
35 *vacuo*. The residue was then purified on silica gel using
5% methanol in DCM as eluent to provide pure Boc methyl
ester.

5 vacuo. The resulting product was used with no further purification.

10 1 equivalent of the methyl ester was dissolved in THF/H₂O (3/1) and 3 equivalents of LiOH·H₂O were added. The reaction was monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was acidified carefully to pH 2 with concentrated HCl and then concentrated *in vacuo* to remove the THF. The resulting aqueous layer was washed twice with Et₂O and the combined organic layers were 15 washed once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*. The benzoic acid t-butyl ester was used without further purification.

20 1 equivalent of 3-methoxybenzonitrile was placed in a Parr bottle with EtOH, 0.02 equivalents of HCl and 10% (w/w) of 10% Pd on carbon. The vessel was placed in the Parr shaker, charged with 50psi H₂, and shaken for 12 hours. The reaction filtered through a pad of celite and 25 diluted 1:10 with Et₂O. Upon standing over night, fine white needles form. The product was filtered, washed with Et₂O and dried *in vacuo*. The resulting amine hydrochloride salt was then used with out further purification.

30 3 equivalents of the benzoic acid t-butyl ester was coupled to 1 equivalent of the amine hydrochloride salt using 3 equivalents EDC, 1 equivalent of HObt and 3 equivalents of DIPEA in DMA. The reaction was monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was 35 concentrated *in vacuo*. The resulting oil was re suspended in Et₂O and washed twice with 0.1 N H₂SO₄, twice with saturated NaHCO₃, and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated *in*

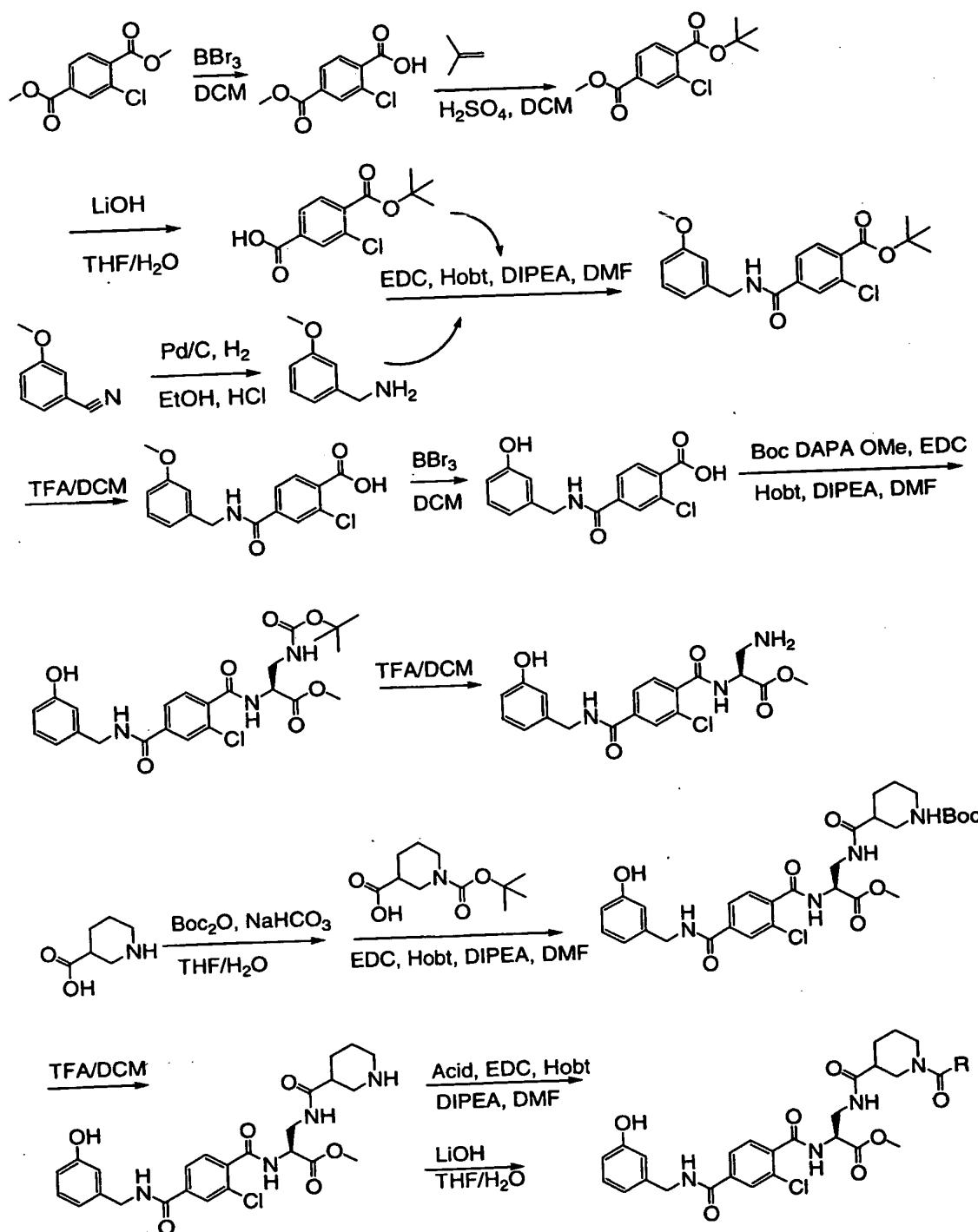
5 wise as a solution in DCM over 30 minutes. The reaction
was warmed to room temperature and stirred until complete
by TLC (DCM/2% HOAc/2% MeOH). The solution was poured
onto ice, and the ice was allowed to melt. The mixture
was then partitioned with EtOAc and concentrated *in*
10 *vacuo*. This product was dissolved in H₂O with the addition
of saturated NaHCO₃ until the pH remained above 8. This
solution was partitioned one time with and equal volume
of DCM to remove unreacted diester. The basic solution
was acidified at 0°C. with concentrated HCl to pH = 1-
15 1.5, and precipitate was extracted twice with equal
volumes of EtOAc. The organics were partitioned once
with brine and dried over MgSO₄, filtered and concentrated
in vacuo. Product was 7:1 of the correct regioisomer by
HPLC.

20 The monoester was dissolved in DCM and transferred to a
pre-weighed Parr flask containing a stirring bar. The
flask was cooled to -5°C with a dry ice/alcohol bath
under nitrogen. Once cool, ~30 equivalents of isobutylene
25 was pumped into solution with stirring. 2.1 equivalents
of concentrated sulfuric acid was added and the flask was
sealed with a wired rubber stopper and allowed to warm to
room temperature with stirring. The solution was stirred
until clarification (1-2 days). Once the solution was
30 clear, it was cooled to 0°C in an ice bath. The stopper
was removed and the excess isobutylene was blown off with
nitrogen bubbling. Saturated NaHCO₃ was added to
neutralize the acid and the mixture was concentrated *in*
35 *vacuo* until no DCM remained. The solution was then
partitioned into EtOAc. The organics were partitioned
twice with dilute HCl, twice with saturated NaHCO₃, once
with brine, dried over MgSO₄, filtered and concentrated *in*

5

EXAMPLE 6

Synthesis of compounds 29, 30



15

1 equivalent of dimethyl 2- chloroterephthalic acid was dissolved in DCM and cooled to -5°C in an ice/acetone bath under nitrogen. 1 equivalent of BBr_3 was added drop

5 used without further purification) example 26, cyclohexanecarboxylic acid; example 27, isonipecotic acid; example 28, D,L-pipecolinic acid; example 31, nipecotic acid; 2 equivalents of EDC, 1 equivalent of Hobt and 3 equivalents of DIPEA were dissolved DMA. The
10 reaction was stirred at room temperature and monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was concentrated *in vacuo*. The resulting oil was re suspended in Et₂O and washed twice with 0.1 N H₂SO₄, twice with saturated NaHCO₃, and once with brine. The organic layer
15 was then dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was then purified on silica gel using 5% methanol in DCM as eluent to provide pure methyl ester.

20 1 equivalent of the resultant methyl ester was dissolved in THF/H₂O (3/1) and 3 equivalents of LiOH·H₂O was added. The reaction was monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was acidified to pH 2 with 1M HCl and then concentrated *in vacuo*. The resulting solid was
25 re suspended in Et₂O and washed twice with 0.1 M HCl and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*.

30 Where appropriate the Boc protected residue was dissolved in a solution of TFA in DCM (1:1). After 20 minutes, the reaction was concentrated *in vacuo*. The resulting oil was dissolved in toluene and then re concentrated *in vacuo*. The resulting acid was then purified by reverse phase HPLC, verified by electrospray mass spectrometry and
35 lyophilized to a powder.

WO 02/059114

5 filtrate was then passed over a plug of silica gel and
concentrated in vacuo to afford pure benzoic acid.

10 1 equivalent of the benzoic acid, 2 equivalents of
commercially available β -Boc-diaminopropionic acid
methyl ester, 2 equivalents of EDC, 1 equivalent of HObt
and 3 equivalents of DIPEA were dissolved DMA. The
reaction was stirred at room temperature and monitored by
TLC (9/1 DCM/MeOH). Upon completion, the mixture was
concentrated in vacuo. The resulting oil was re suspended
15 in Et₂O and washed twice with 0.1 N H₂SO₄, twice with
saturated NaHCO₃, and once with brine. The organic layer
was then dried over MgSO₄, filtered and concentrated in
vacuo. The residue was then purified on silica gel using
20 5% methanol in DCM as eluent to provide pure methyl
ester.

25 The Boc protected amine was dissolved in a solution of
TFA in DCM (1:1). After 20 minutes, the reaction was
concentrated in vacuo. The resulting oil was dissolved in
toluene and then re concentrated in vacuo. 1 equivalent
of this amine, 2 equivalents of the appropriate
commercially available carboxylic acid ((N-Boc acids were
purchased where available. Other acids were purchased as
the free amine and Boc protected by the following
30 procedure: The amine was dissolved in a 3:2 THF/H₂O
solution. 1.1 equivalents of solid NaHCO₃ and 1.1
equivalents of Boc₂O were added and the mixture was
stirred overnight. The reaction was concentrated to
remove the THF, and the resulting aqueous layer was
35 partitioned with hexanes. The aqueous layer was then
acidified to pH 2 with 1N HCl and then partitioned twice
with EtOAc. The combined organic layers were dried over
MgSO₄ and concentrated in vacuo. The resulting product was

5 (w/w) of 10% Pd on carbon. The vessel was placed in the Parr shaker, charged with 50psi H₂, and shaken for 12 hours. The reaction filtered through a pad of celite and diluted 1:10 with Et₂O. Upon standing over night, fine white needles form. The product was filtered, washed with
10 Et₂O and dried *in vacuo*. The resulting amine hydrochloride salt was then used with out further purification.

3 equivalents of the benzoic acid t-butyl ester was coupled to 1 equivalent of the amine hydrochloride salt
15 using 3 equivalents EDC, 1 equivalent of Hobt and 3 equivalents of DIPEA in DMA. The reaction was monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was concentrated *in vacuo*. The resulting oil was re suspended in Et₂O and washed twice with 0.1 N H₂SO₄, twice with
20 saturated NaHCO₃, and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*. The product was then purified on silica gel using 5% methanol in DCM as eluent to provide pure t-butyl ester.

25 The t-butyl ester was dissolved in a solution of TFA in DCM (1:1). After 20 minutes, the reaction was concentrated *in vacuo*. The resulting oil was dissolved in toluene and then concentrated *in vacuo* twice.

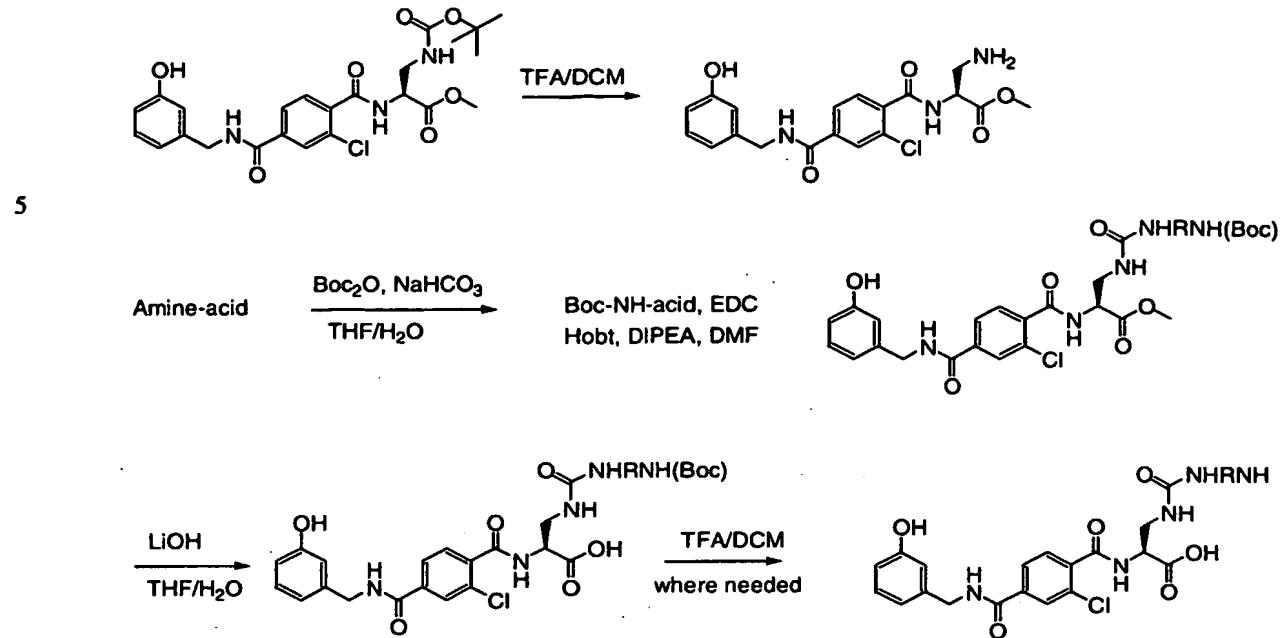
30 The resulting compound was dissolved in DCM and cooled to -5°C in an ice/acetone bath under nitrogen. 2 equivalents of BBr₃ were added drop wise as a solution in DCM over 30 minutes. The reaction was warmed to room temperature and stirred until complete by TLC (DCM/2% HOAc/2% MeOH). The solution was poured onto ice, and the ice was allowed to melt. The mixture was then partitioned twice with EtOAc and the combined organic layers were dried over MgSO₄. The
35

WO 02/059114

5 The monoester was dissolved in DCM and transferred to a
pre-weighed Parr flask containing a stirring bar. The
flask was cooled to -5°C with a dry ice/alcohol bath
under nitrogen. Once cool, ~30 equivalents of isobutylene
was pumped into solution with stirring. 2.1 equivalents
10 of concentrated sulfuric acid was added and the flask was
sealed with a wired rubber stopper and allowed to warm to
room temperature with stirring. The solution was stirred
until clarification (1-2 days). Once the solution was
clear, it was cooled to 0°C in an ice bath. The stopper
15 was removed and the excess isobutylene was blown off with
nitrogen bubbling. Saturated NaHCO₃ was added to
neutralize the acid and the mixture was concentrated in
vacuo until no DCM remained. The solution was then
partitioned into EtOAc. The organics were partitioned
20 twice with dilute HCl, twice with saturated NaHCO₃, once
with brine, dried over MgSO₄, filtered and concentrated in
vacuo. The resulting product was used with no further
purification.

25 1 equivalent of the methyl ester was dissolved in THF/H₂O
(3/1) and 3 equivalents of LiOH·H₂O was added. The
reaction was monitored by TLC (9/1 DCM/MeOH). Upon
completion, the mixture was acidified carefully to pH 2
with concentrated HCl and then concentrated in vacuo to
remove the THF. The resulting aqueous layer was washed
30 twice with Et₂O and the combined organic layers were
washed once with brine. The organic layer was then dried
over MgSO₄, filtered and concentrated in vacuo. The
benzoic acid t-butyl ester was used without further
purification.

35 1 equivalent of 3-methoxybenzonitrile was placed in a
Parr bottle with EtOH, 0.02 equivalents of HCl and 10%



1 equivalent of dimethyl 2- chloroterephthalic acid was dissolved in DCM and cooled to -5°C in an ice/acetone bath under nitrogen. 1 equivalent of BBr_3 was added drop wise as a solution in DCM over 30 minutes. The reaction was warmed to room temperature and stirred until complete by TLC (DCM/2% HOAc/2% MeOH). The solution was poured onto ice, and the ice was allowed to melt. The mixture was then partitioned with EtOAc and concentrated *in vacuo*. This product was dissolved in H_2O with the addition of saturated NaHCO_3 until the pH remained above 8. This solution was partitioned one time with an equal volume of DCM to remove unreacted diester. The basic solution was acidified at 0°C. with concentrated HCl to pH = 1-1.5, and precipitate was extracted twice with equal volumes of EtOAc. The organics were partitioned once with brine and dried over MgSO_4 , filtered and concentrated *in vacuo*. Product was 7:1 of the correct regioisomer by HPLC.

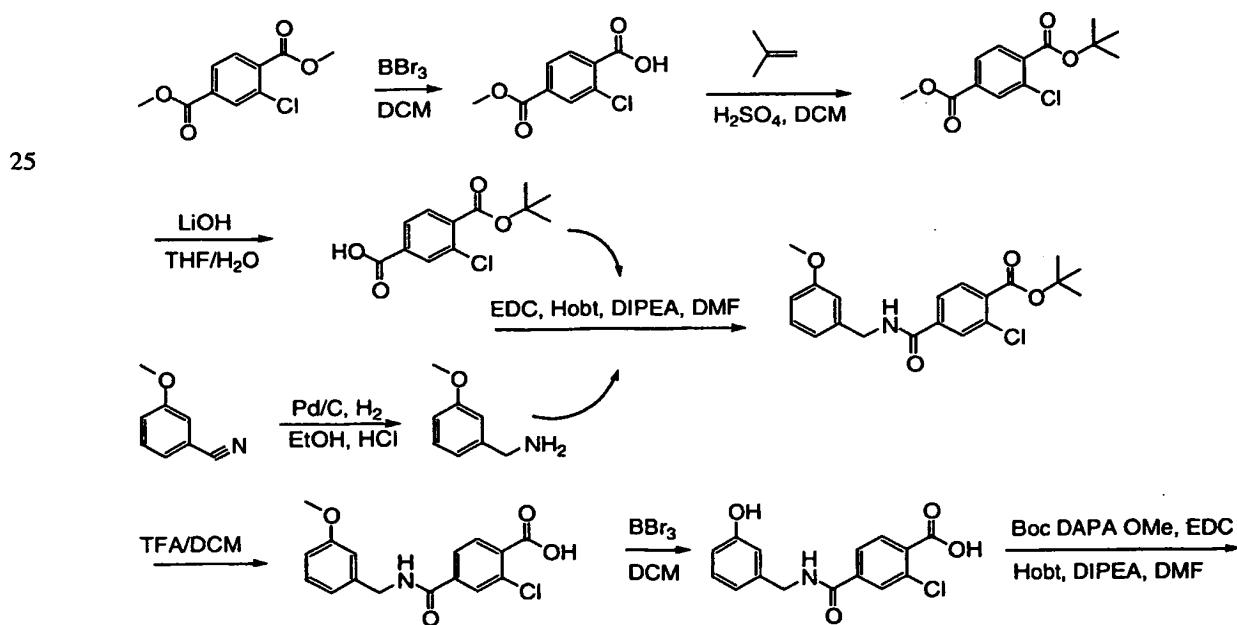
5

1 equivalent of the resultant methyl ester was dissolved in THF/H₂O (3/1) and 3 equivalents of LiOH·H₂O was added. The reaction was monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was acidified to pH 2 with 1M HCl and then concentrated *in vacuo*. The resulting solid was re suspended in Et₂O and washed twice with 0.1 M HCl and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*.

The Boc, silyl residue was dissolved in a solution of TFA in DCM (1:1) with 3 equivalents of TBAF. After 20 minutes, the reaction was concentrated *in vacuo*. The resulting oil was dissolved in toluene and then reconcentrated *in vacuo*. The resulting acid was then purified by reverse phase HPLC, verified by electrospray mass spectrometry and lyophilized to a powder.

EXAMPLE 5

Synthesis of compounds 26-28, 31



5 dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was then purified on silica gel using 5% methanol in DCM as eluent to provide pure methyl ester.

10 The Boc protected amine was dissolved in a solution of TFA in DCM (1:1). After 20 minutes, the reaction was concentrated *in vacuo*. The resulting oil was dissolved in toluene and then reconcentrated *in vacuo*. 1 equivalent of this amine, 2 equivalents of the appropriate commercially available carboxylic acid ((N-Boc acids were purchased 15 where available. Other acids were purchased as the free amine and Boc protected by the following procedure: The amine was dissolved in a 3:2 THF/H₂O solution. 1.1 equivalents of solid NaHCO₃ and 1.1 equivalents of Boc₂O were added and the mixture was stirred overnight. The 20 reaction was concentrated to remove the THF, and the resulting aqueous layer was partitioned with hexanes. The aqueous layer was then acidified to pH 2 with 1N HCl and then partitioned twice with EtOAc. The combined organic layers were dried over MgSO₄ and concentrated *in vacuo*. 25 The resulting product was used without further purification) example 22, N-Boc-L-proline; example 23, N-Boc-D-proline; example 24, Boc-L-thiazolidine-4-carboxylic acid; example 25, D-hydroxy proline; 2 equivalents of EDC, 1 equivalent of Hobt and 3 30 equivalents of DIPEA were dissolved DMA. The reaction was stirred at room temperature and monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was concentrated *in vacuo*. The resulting oil was re suspended in Et₂O and washed twice with 0.1 N H₂SO₄, twice with saturated 35 NaHCO₃, and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was then purified on silica gel using 5% methanol in DCM as eluent to provide pure methyl ester.

5 1 equivalent of the aryl alkyne was dissolved in MeOH and
the solution was degassed by passing N₂ through a pipette
and into the solution for 10 minutes. The 5% Rh/Al₂O₃ was
added, one balloon-full of hydrogen was passed through
the solution, and the reaction was stirred under an
10 atmosphere of H₂ (using a balloon) for 7 hours, after
which the reaction was filtered through a pad of celite
and concentrated *in vacuo*. The residue was purified by
silica gel flash chromatography (gradient elution, using
Et₂O to EtOAc) to provide the pure product.

15 2.3 equivalents of lithium iodide was added to 1
equivalent of the methyl ester in pyridine, and the
mixture heated at reflux for 8 hours. The reaction was
concentrated *in vacuo* and the residue was partitioned
20 between EtOAc and 1N HCl. The aqueous layer was extracted
three times with EtOAc, and the combined organic layers
were washed with 1M NaHCO₃, dried over MgSO₄ and
concentrated *in vacuo*. The residue was dissolved in NMM
and the solution concentrated *in vacuo*. The residue was
25 taken up in DCM and then washed three times with 1N HCl.
The organic layer was dried over MgSO₄ and concentrated *in
vacuo* to provide the benzoic acid in high enough purity
to be used without further purification.

30 1 equivalent of the acid, 2 equivalents of commercially
available β - Boc- diaminopropionic acid methyl ester, 2
equivalents of EDC, 1 equivalent of Hobt and 3
equivalents of DIPEA were dissolved DMA. The reaction was
stirred at room temperature and monitored by TLC (9/1
35 DCM/MeOH). Upon completion, the mixture was concentrated
in vacuo. The resulting oil was re suspended in Et₂O and
washed twice with 0.1 N H₂SO₄, twice with saturated
NaHCO₃, and once with brine. The organic layer was then

5 quenched by adding to a separatory funnel containing Et₂O and 0.35M NaHSO₄. The layers were separated. The aqueous layer was extracted three times with ethyl ether. The combined organic layers were washed twice with 1N HCl, saturated aqueous NaHCO₃, and over MgSO₄, filtered through
10 a plug of silica gel, and concentrated in vacuo. No further purification of the aldehyde was necessary.

A solution of 1 equivalent of the protected aldehyde in THF was cooled to -78°C and 1.1 equivalents of 0.5M ethynylmagnesium bromide/THF was added. After stirring the reaction at room temperature for 3 hours, it was diluted with Et₂O and washed twice with 10% citric acid. The combined aqueous layers were back-extracted once with Et₂O. The combined organic layers were washed twice with 20 saturated aqueous NaHCO₃, dried over MgSO₄ and concentrated in vacuo. The residue was purified by silica gel flash chromatography (4:1 to 3:2 hexane/Et₂O) to provide the pure alkyne.

25 1 equivalent of the aryl iodide methyl ester was dissolved in EtOAc and the solution was degassed by passing N₂ through a pipette and into the solution for 10 minutes. 1.25 equivalents of the alkyne was added, followed by 0.02 equivalents of dichlorobis(triphenylphosphine)palladium(II), 0.04 equivalents of CuI and 5 equivalents TEA. The reaction was stirred for 14 hours, diluted with EtOAc, washed twice with 5% Na₂•EDTA, brine and then dried over MgSO₄ and concentrated in vacuo. The residue was purified by 30 silica gel flash chromatography (gradient elution, using Et₂O to EtOAc) to provide the pure aryl alkyne.

5 vacuo. The residue was purified by silica gel flash chromatography (95:5 hexane/Et₂O) to provide the pure aryl iodide methyl ester.

10 1.3 equivalents of DIPEA was added to a heterogeneous mixture of 1 equivalent of 3-hydroxybenzoic acid, 1.3 equivalents of N, O-dimethylhydroxylamine hydrochloride, 1.3 equivalents of HOBt and 1.3 equivalents of EDC stirring in DMF. All solids eventually dissolved as the mixture was stirred at room temperature for 28 hours.

15 After concentrating the mixture, the residue was partitioned between Et₂O and H₂O. The aqueous layer was extracted three times with Et₂O and the combined organic layers were dried over MgSO₄, and concentrated *in vacuo*. The residue was purified by silica gel flash chromatography (Et₂O) to provide the pure hydroxamate.

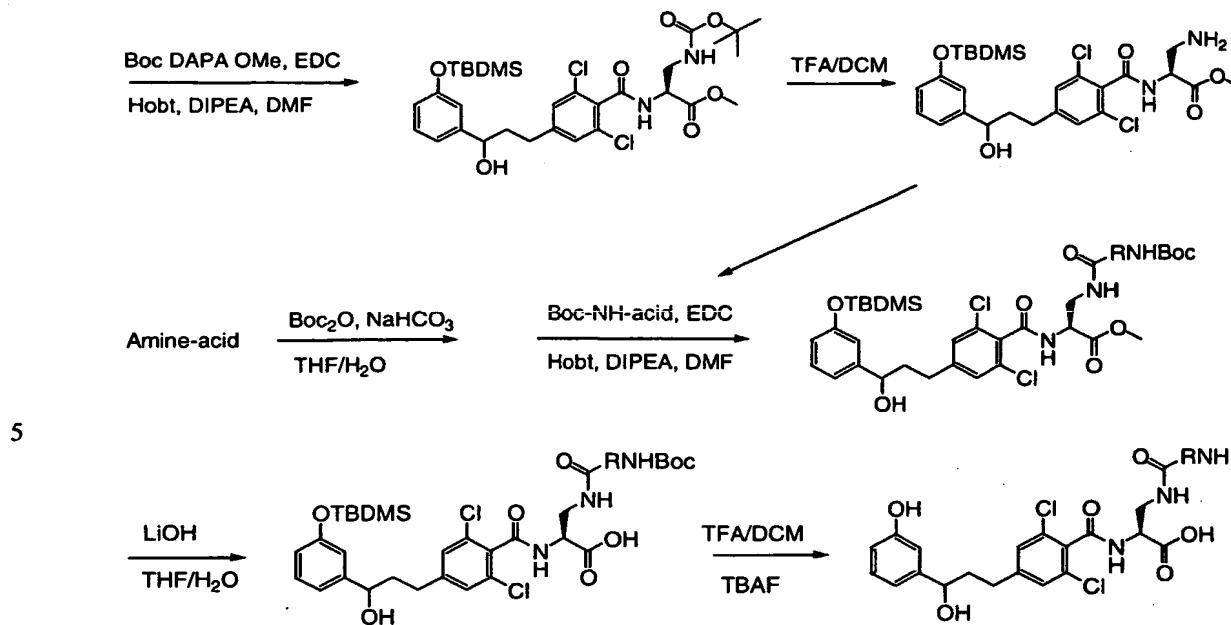
20 1 equivalent of the hydroxamate, 2.2 equivalents of t-butyldimethyl silyl chloride and 3 equivalents of imidazole were dissolved in DMF and stirred at room temperature. The reaction was monitored by TLC (9/1 DCM/MeOH). Upon reaction completion, the mixture was concentrated *in vacuo*. The resulting oil was re suspended in Et₂O and washed twice with saturated NaHCO₃, and once with brine. The organic layer was then dried over MgSO₄, 25 filtered and concentrated *in vacuo*. The product was then used with out further purification.

30 To a stirred -78°C solution of 1 equivalent of the protected hydroxamate in THF was added a solution of 1.2 equivalents of 1.5 M DIBAL in toluene drop wise. The reaction mixture was stirred for an additional 3 hours at -78°C or until TLC showed clean formation of product, with only a trace of starting material. The reaction was

5 was purified by silica gel flash chromatography (9:1 hexane/Et₂O) to provide the pure triflate.

To a stirring solution of 1 equivalent of the triflate in a 2/1 mixture of DMF/MeOH was added 0.15 equivalents of 10 1, 3-bis(diphenylphosphino)-propane and 2.5 equivalents of TEA. Carbon monoxide gas was bubbled through this solution for 15 minutes, then 0.15 equivalents of Pd(OAc)₂ was added and the reaction was stirred at 70°C for 5-7 hours under an atmosphere of CO (using a balloon 15 filled with CO). The reaction was then concentrated in vacuo, and the residue was partitioned between Et₂O and H₂O. The aqueous layer was extracted twice with Et₂O and the combined organic layers were dried over MgSO₄, filtered through a plug of silica gel and concentrated in 20 vacuo. The residue was purified by silica gel flash chromatography (9:1:0.02 hexane/DCM/Et₂O) to provide the pure methyl ester.

25 1 equivalent of the Boc-aniline was dissolved in methanol and the solution saturated with HCl. The reaction was heated at 50°C for 3h, then concentrated in vacuo. The pale yellow solid was heated in 35% H₂SO₄ until complete dissolution occurred. Upon cooling the mixture by the addition of ice H₂O the amine bisulfate precipitated. The 30 reaction flask was cooled in an ice bath and the mixture stirred vigorously while 1.1 equivalents of sodium nitrite in H₂O was added drop wise. The reaction was stirred at 0°C for another 1.5 hours. An aqueous solution of 35 10 equivalents of KI was added, followed immediately with 17 equivalents CuI. The reaction was stirred at room temperature for 14 hours, then extracted 3 times with Et₂O. The combined organic layers were washed with 1M NaHCO₃, brine, and dried over MgSO₄, then concentrated in



1 equivalent of 4-amino-2, 6-dichlorophenol was dissolved in a 3:2 THF/H₂O solution. 1.1 equivalents of solid NaHCO₃ and 1.1 equivalents of Boc₂O were added and the solution was stirred overnight. The reaction was concentrated, and the residue was partitioned between H₂O and Et₂O. The aqueous layer was extracted with Et₂O and the combined organic layers were dried over MgSO₄ and concentrated *in vacuo* to a solid. Recrystallization out of Et₂O/hexane provided pure product.

10

1 equivalent of the phenol was dissolved in DCM containing 2.6 equivalents of 2, 6-lutidine and the mixture was cooled to -78°C. After adding 1.25 equivalents of triflic anhydride the stirring reaction was allowed to warm to room temperature overnight. The reaction was then concentrated, and the residue was partitioned between Et₂O and H₂O. The aqueous layer was extracted with Et₂O and the combined organic layers were dried over MgSO₄ and concentrated *in vacuo*. The residue

20

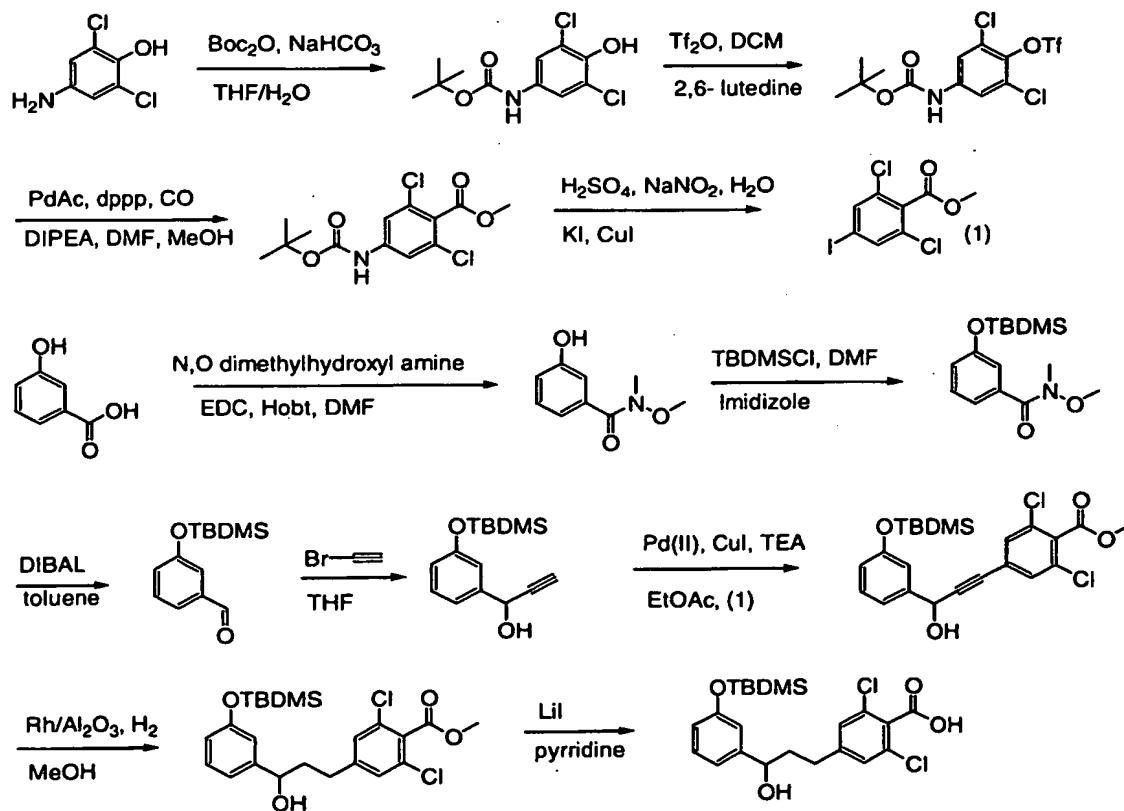
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5 The reaction was monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was acidified to pH 2 with 1M HCl and then concentrated *in vacuo*. The resulting solid was re suspended in Et₂O and washed twice with 0.1 M HCl and once with brine. The organic layer was then dried over 10 MgSO₄, filtered and concentrated *in vacuo*.

The Boc protected residue was dissolved in a solution of TFA in DCM (1:1). After 20 minutes, the reaction was concentrated *in vacuo*. The resulting oil was dissolved in 15 toluene and then reconcentrated *in vacuo*. The resulting acid was then purified by reverse phase HPLC, verified by electrospray mass spectrometry and lyophilized to a powder.

20

EXAMPLE 4 Synthesis of compounds 22-25



25

5

The Boc protected amine was dissolved in a solution of TFA in DCM (1:1). After 20 minutes, the reaction was concentrated *in vacuo*. The resulting oil was dissolved in toluene and then reconcentrated *in vacuo*. 1 equivalent of this amine, 2 equivalents of the appropriate commercially available carboxylic acid ((N-Boc acids were purchased where available. Other acids were purchased as the free amine and Boc protected by the following procedure: The amine was dissolved in a 3:2 THF/H₂O solution. 1.1 equivalents of solid NaHCO₃ and 1.1 equivalents of Boc₂O were added and the mixture was stirred overnight. The reaction was concentrated to remove the THF, and the resulting aqueous layer was partitioned with hexanes. The aqueous layer was then acidified to pH 2 with 1N HCl and then partitioned twice with EtOAc. The combined organic layers were dried over MgSO₄ and concentrated *in vacuo*. The resulting product was used without further purification) example 18, N-Boc-D-proline; example 19, N-Boc-L-proline; example 20, Boc-L-thiazolidine-4-carboxylic acid; example 21, isonipecotic acid; 2 equivalents of EDC, 1 equivalent of Hobt and 3 equivalents of DIPEA were dissolved DMA. The reaction was stirred at room temperature and monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was concentrated *in vacuo*. The resulting oil was re suspended in Et₂O and washed twice with 0.1 N H₂SO₄, twice with saturated NaHCO₃, and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was then purified on silica gel using 5% methanol in DCM as eluent to provide pure methyl ester.

1 equivalent of the resultant methyl ester was dissolved in THF/H₂O (3/1) and 3 equivalents of LiOH•H₂O was added.

5 added, one balloon-full of hydrogen was passed through
the solution, and the reaction was stirred under an
atmosphere of H₂ (using a balloon) for 7 hours, after
which the reaction was filtered through a pad of celite
10 and concentrated *in vacuo*. The residue was purified by
silica gel flash chromatography (gradient elution, using
Et₂O to EtOAc) to provide the pure product.

2.3 equivalents of lithium iodide was added to 1
equivalent of the methyl ester in pyridine, and the
15 mixture heated at reflux for 8 hours. The reaction was
concentrated *in vacuo* and the residue was partitioned
between EtOAc and 1N HCl. The aqueous layer was extracted
three times with EtOAc, and the combined organic layers
were washed with 1M NaHCO₃, dried over MgSO₄ and
20 concentrated *in vacuo*. The residue was dissolved in NMM
and the solution concentrated *in vacuo*. The residue was
taken up in DCM and then washed three times with 1N HCl.
The organic layer was dried over MgSO₄ and concentrated *in
25 vacuo* to provide the benzoic acid in high enough purity
to be used without further purification.

1 equivalent of the acid, 2 equivalents of commercially
available β -Boc- diaminopropionic acid methyl ester, 2
equivalents of EDC, 1 equivalent of Hobt and 3
30 equivalents of DIPEA were dissolved DMA. The reaction was
stirred at room temperature and monitored by TLC (9/1
DCM/MeOH). Upon completion, the mixture was concentrated
in vacuo. The resulting oil was re suspended in Et₂O and
washed twice with 0.1 N H₂SO₄, twice with saturated
35 NaHCO₃, and once with brine. The organic layer was then
dried over MgSO₄, filtered and concentrated *in vacuo*. The
residue was then purified on silica gel using 5% methanol
in DCM as eluent to provide pure methyl ester.

5 Et₂O. The combined organic layers were washed with 1M NaHCO₃, brine, and dried over MgSO₄, then concentrated *in vacuo*. The residue was purified by silica gel flash chromatography (95:5 hexane/Et₂O) to provide the pure aryl iodide methyl ester.

10 A solution of 1 equivalent of 3-Chlorobenzaldehyde in THF was cooled to -78°C and 1.1 equivalents of 0.5M ethynylmagnesium bromide/THF was added. After stirring the reaction at room temperature for 3 hours, it was 15 diluted with Et₂O and washed twice with 10% citric acid. The combined aqueous layers were back-extracted once with Et₂O. The combined organic layers were washed twice with saturated aqueous NaHCO₃, dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by silica 20 gel flash chromatography (4:1 to 3:2 hexane/Et₂O) to provide the pure alkyne.

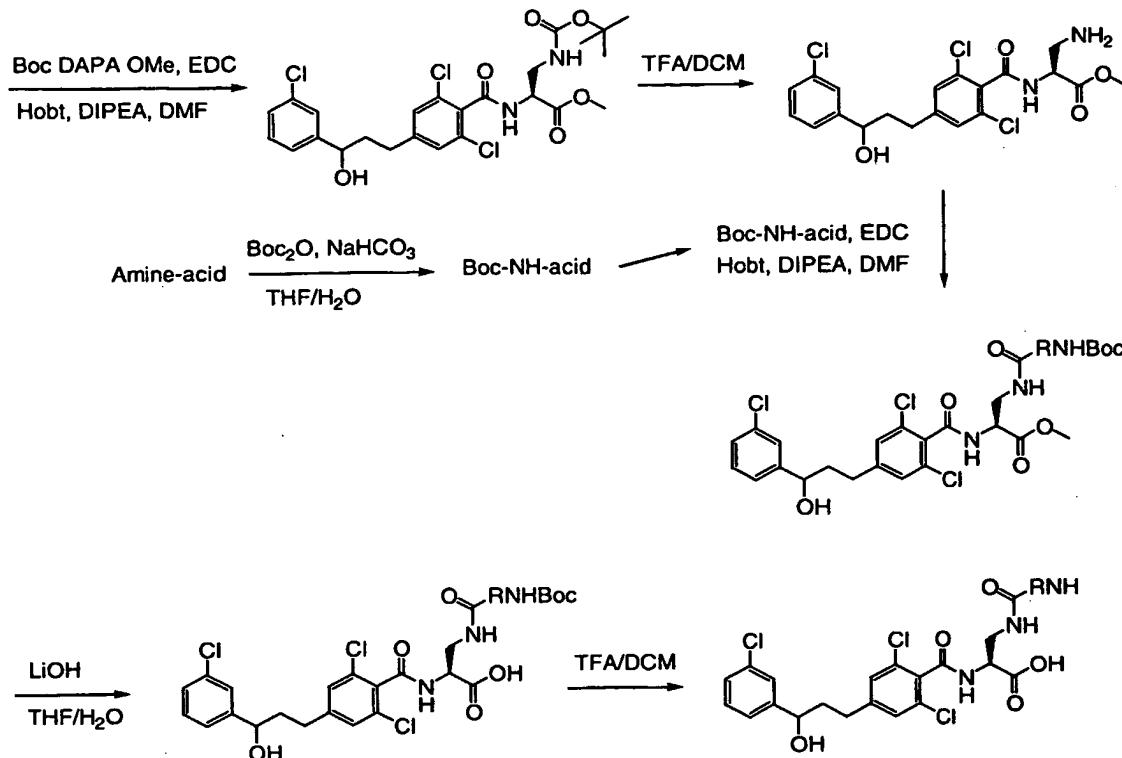
1 equivalent of the aryl iodide methyl ester was dissolved in EtOAc and the solution was degassed by 25 passing N₂ through a pipette and into the solution for 10 minutes. 1.25 equivalents of the alkyne was added, followed by 0.02 equivalents of dichlorobis(triphenylphosphine)palladium(II), 0.04 equivalents of CuI and 5 equivalents TEA. The reaction 30 was stirred for 14 hours, diluted with EtOAc, washed twice with 5% Na₂•EDTA, brine and then dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by silica gel flash chromatography (gradient elution, using Et₂O to EtOAc) to provide the pure aryl alkyne.

35 1 equivalent of the aryl alkyne was dissolved in MeOH and the solution was degassed by passing N₂ through a pipette and into the solution for 10 minutes. The 5% Rh/Al₂O₃ was

5 extracted with Et₂O and the combined organic layers were dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by silica gel flash chromatography (9:1 hexane/Et₂O) to provide the pure triflate.

10 To a stirring solution of 1 equivalent of the triflate in a 2/1 mixture of DMF/MeOH was added 0.15 equivalents of 1, 3-bis(diphenylphosphino)-propane and 2.5 equivalents of TEA. Carbon monoxide gas was bubbled through this solution for 15 minutes, then 0.15 equivalents of 15 Pd(OAc)₂ was added and the reaction was stirred at 70°C for 5-7 hours under an atmosphere of CO (using a balloon filled with CO). The reaction was then concentrated *in vacuo*, and the residue was partitioned between Et₂O and H₂O. The aqueous layer was extracted twice with Et₂O and 20 the combined organic layers were dried over MgSO₄, filtered through a plug of silica gel and concentrated *in vacuo*. The residue was purified by silica gel flash chromatography (9:1:0.02 hexane/DCM/Et₂O) to provide the pure methyl ester.

25 1 equivalent of the Boc-aniline was dissolved in methanol and the solution saturated with HCl. The reaction was heated at 50°C for 3h, then concentrated *in vacuo*. The pale yellow solid was heated in 35% H₂SO₄ until complete 30 dissolution occurred. Upon cooling the mixture by the addition of ice H₂O the amine bisulfate precipitated. The reaction flask was cooled in an ice bath and the mixture stirred vigorously while 1.1 equivalents of sodium nitrite in H₂O was added drop wise. The reaction was 35 stirred at 0°C for another 1.5 hours. An aqueous solution of 10 equivalents of KI was added, followed immediately with 17 equivalents CuI. The reaction was stirred at room temperature for 14 hours, then extracted 3 times with



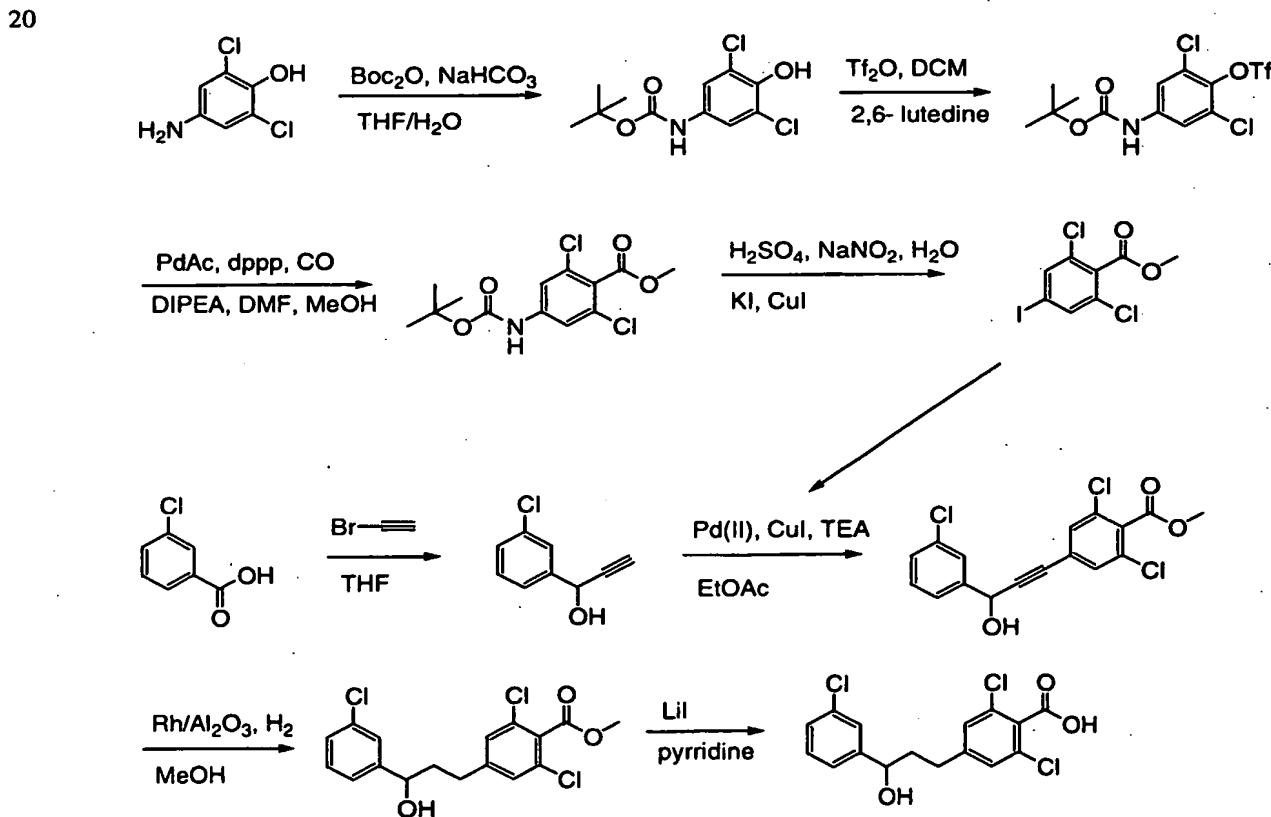
1 equivalent of 4-amino-2,6-dichlorophenol was dissolved in a 3:2 THF/H₂O solution. 1.1 equivalents of solid NaHCO₃ and 1.1 equivalents of Boc₂O were added and the solution was stirred overnight. The reaction was concentrated, and the residue was partitioned between H₂O and Et₂O. The aqueous layer was extracted with Et₂O and the combined organic layers were dried over MgSO₄ and concentrated *in vacuo* to a solid. Recrystallization out of Et₂O/hexane provided pure product.

1 equivalent of the phenol was dissolved in DCM containing 2.6 equivalents of 2, 6-lutidine and the mixture was cooled to -78°C. After adding 1.25 equivalents of triflic anhydride the stirring reaction was allowed to warm to room temperature overnight. The reaction was then concentrated, and the residue was partitioned between Et₂O and H₂O. The aqueous layer was

5 and then concentrated *in vacuo*. The resulting solid was re suspended in Et₂O and washed twice with 0.1 M HCl and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*.

10 Where appropriate the Boc protected residue was dissolved in a solution of TFA in DCM (1:1). After 20 minutes, the reaction was concentrated *in vacuo*. The resulting oil was dissolved in toluene and then reconcentrated *in vacuo*. The resulting acid was then purified by reverse phase HPLC, verified by electrospray mass spectrometry and lyophilized to a powder.

EXAMPLE 3 Synthesis of compounds 18-21



reaction was concentrated to remove the THF, and the resulting aqueous layer was partitioned with hexanes. The aqueous layer was then acidified to pH 2 with 1N HCl and then partitioned twice with EtOAc. The combined organic layers were dried over MgSO₄ and concentrated in vacuo. The resulting product was used without further purification) compound 1 D,L-pipecolinic acid; compound 2, nipecotic acid; compound 3, isonipecotic acid; compound 4, N-Boc-L-proline; compound 5, N-Boc-D-proline; compound 6, Boc-L-thiazolidine-4-carboxylic acid; compound 7, N-Boc-L-pyroglutamic acid; compound 8, N-Boc-D-pyroglutamic acid; compound 9, L-pipecolinic acid; compound 10, D-cis-4-hydroxyproline; compound 11, L-cis-4-hydroxyproline; compound 12, D-hydroxyproline; compound 13, (2S, 3S)-3-methylpyrrolidine-2-carboxylic acid; compound 14, N-Boc-L-hydroxyproline; compound 15, Boc-D-thiazolidine-4-carboxylic acid; compound 41, L-3-hydroxyproline; compound 43, trans-3-azabicyclo[3.1.0]-hexane-2-carboxylic acid), 2 equivalents of EDC, 1 equivalent of Hobt and 3 equivalents of DIPEA were dissolved DMA. The reaction was stirred at room temperature and monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was concentrated in vacuo. The resulting oil was re suspended in Et₂O and washed twice with 0.1 N H₂SO₄, twice with saturated NaHCO₃, and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated in vacuo. The residue was then purified on silica gel using 5% methanol in DCM as eluent to provide pure methyl ester.

35 1 equivalent of the resultant methyl ester was dissolved in THF/H₂O (3/1) and 3 equivalents of LiOH·H₂O was added. The reaction was monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was acidified to pH 2 with 1M HCl

5 between EtOAc and 1N HCl. The aqueous layer was extracted
three times with EtOAc, and the combined organic layers
were washed with 1M NaHCO₃, dried over MgSO₄ and
concentrated *in vacuo*. The residue was dissolved in NMM
and the solution concentrated *in vacuo*. The residue was
10 taken up in DCM and then washed three times with 1N HCl.
The organic layer was dried over MgSO₄ and concentrated *in
vacuo* to provide the benzoic acid in high enough purity
to be used without further purification.

15 1 equivalent of the acid, 2 equivalents of commercially
available β - Boc- diaminopropionic acid methyl ester, 2
equivalents of EDC, 1 equivalent of Hobt and 3
equivalents of DIPEA were dissolved DMA. The reaction was
stirred at room temperature and monitored by TLC (9/1
20 DCM/MeOH). Upon completion, the mixture was concentrated
in vacuo. The resulting oil was re suspended in Et₂O and
washed twice with 0.1 N H₂SO₄, twice with saturated
NaHCO₃, and once with brine. The organic layer was then
dried over MgSO₄, filtered and concentrated *in vacuo*. The
25 residue was then purified on silica gel using 5% methanol
in DCM as eluent to provide pure methyl ester.

The Boc protected amine was dissolved in a solution of
TFA in DCM (1:1). After 20 minutes, the reaction was
30 concentrated *in vacuo*. The resulting oil was dissolved in
toluene and then reconcentrated *in vacuo*. 1 equivalent of
this amine, 2 equivalents of the appropriate commercially
available carboxylic acid ((N-Boc acids were purchased
where available. Other acids were purchased as the free
35 amine and Boc protected by the following procedure: The
amine was dissolved in a 3:2 THF/H₂O solution. 1.1
equivalents of solid NaHCO₃ and 1.1 equivalents of Boc₂O
were added and the mixture was stirred overnight. The

WO 02/059114

5 time 2.5 equivalents of diisopropyl ethyl amine was added. After properly assembling the bomb, it was charged with 300psi CO gas and heated to 70°C with stirring overnight. The bomb was then cooled and vented. The mixture was transferred to a round bottom flask and 10 concentrated in vacuo. The residue was then purified on silica gel using DCM with 1% acetone and 1% TEA as eluent to provide pure methyl ester.

15 The Boc protected amine was dissolved in a solution of TFA in DCM (1:1). After 20 minutes, the reaction was concentrated in vacuo. The resulting oil was dissolved in toluene and then reconcentrated in vacuo. The TFA salt of the amine was dissolved in Et₂O and washed twice with a 20 10% solution of K₂CO₃ in H₂O and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated in vacuo.

25 1 equivalent of the free based amine, 3 equivalents of furylacrylic acid, 3 equivalents of EDC and 1 equivalent of Hobt were dissolved DMA. The reaction was stirred at room temperature and monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was concentrated in vacuo. The resulting oil was re suspended in Et₂O and washed twice with 0.1 N H₂SO₄, twice with saturated NaHCO₃, and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated in vacuo. The residue was 30 then purified on silica gel using 5% methanol in DCM as eluent to provide pure methyl ester.

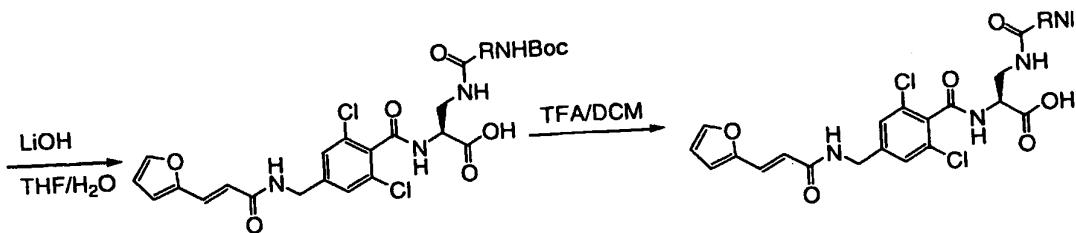
35 2.3 equivalents of lithium iodide was added to 1 equivalent of the methyl ester in pyridine, and the mixture heated at reflux for 8 hours. The reaction was concentrated in vacuo and the residue was partitioned

5 The crude amine residue was dissolved in a 3:2 THF/H₂O solution. 1.1 equivalents of solid NaHCO₃ and 1.1 equivalents of Boc₂O were added and the mixture was stirred overnight. The reaction was concentrated, and the residue was partitioned between H₂O and Et₂O. The aqueous
10 layer was extracted with Et₂O and the combined organic layers were dried over MgSO₄ and concentrated in vacuo to a solid. Recrystallization from hot methanol and H₂O provided pure product.

15 1 equivalent of the Boc protected amine and 1.5 equivalents of 2, 6- lutidine was dissolved, with mild heating when necessary, in DCM in a round bottom flask. Once the starting material had completely dissolved, the mixture was cooled to -78°C under N₂ with a dry ice ethanol bath. Once cool, 2.5 equivalents of triflic anhydride was added and the reaction was allowed to slowly come to room temperature with stirring. The reaction was monitored by TLC and was generally done in 4 hours. Upon completion, the reaction was concentrated in vacuo and the residue partitioned between EtOAc and H₂O.
20 The organic layer was washed twice with 0.1N H₂SO₄, twice with saturated NaHCO₃, once with brine, dried over MgSO₄ and concentrated in vacuo. The residue was then purified on silica gel using DCM as eluent to provide pure
25 triflate.
30

1 equivalent of triflate was dissolved in DMF and MeOH in the glass insert of a high pressure Parr bomb. The starting material was then degassed while stirring with CO for 10 minutes. 0.15 equivalents palladium(II) acetate and 0.15 equivalents of 1, 3- bis(diphenylphosphino) propane were then added and the mixture was then degassed while stirring with CO for another 10 minutes at which

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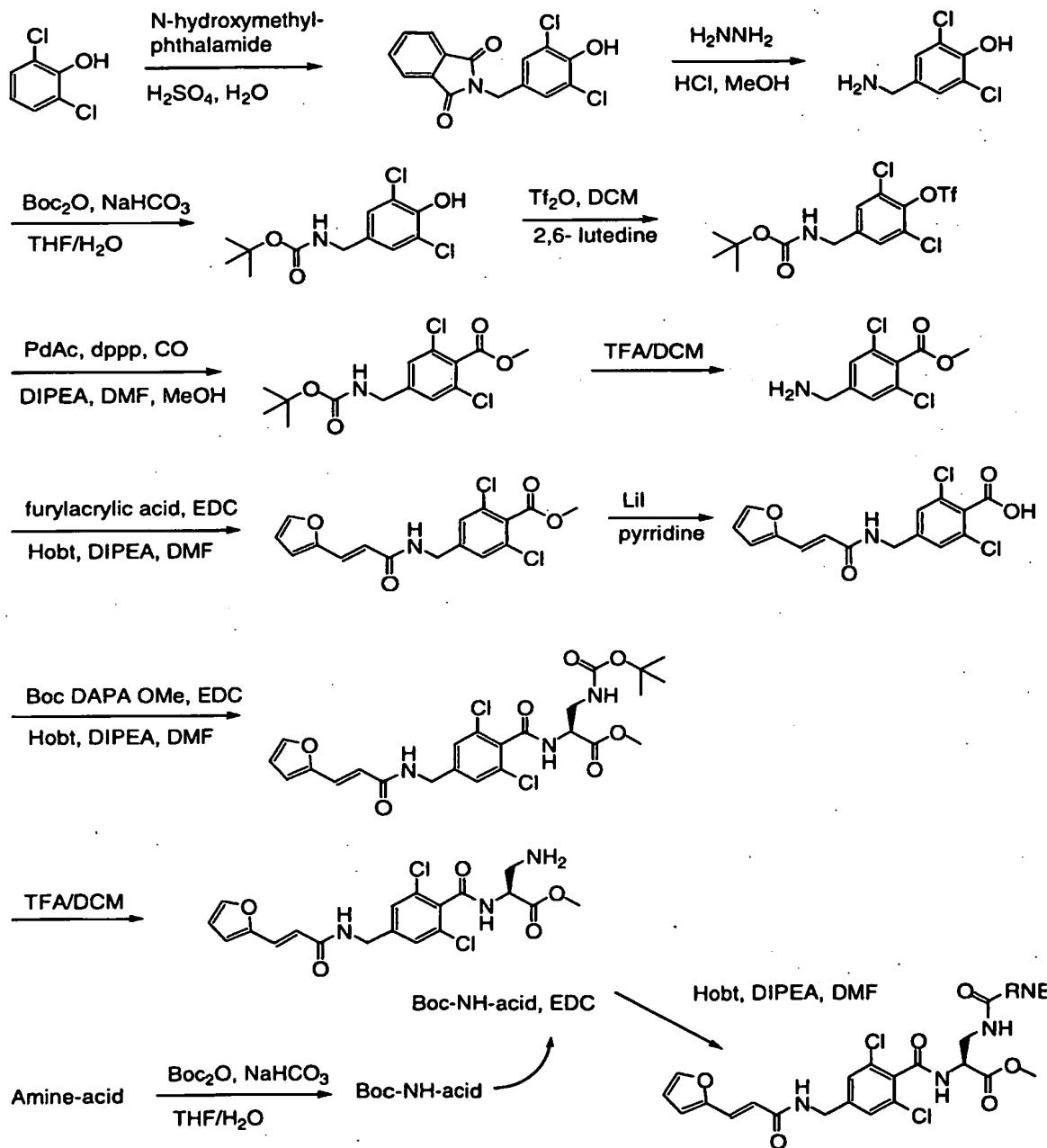
5 A round bottom flask was equipped with an efficient overhead stirrer and charged with concentrated H_2SO_4 (2.7 x volume of H_2O) and H_2O and cooled to $\sim 5^\circ\text{C}$ with an 10 ethanol/ice bath. Once cool, 1 equivalent 2,6 dichloro phenol and 1 equivalent of N-(hydroxymethyl)phthalimide were added with vigorous stirring. The reaction was kept cool for 4 hours and then allowed to warm to room 15 temperature overnight with constant stirring. The reaction generally proceeds to a point where there was just a solid in the round bottom flask. At this point EtOAc and H_2O were added and stirred into the solid. Large chunks were broken up and then the precipitate was filtered and washed with more EtOAc and H_2O . The product 20 was then used without further purification after drying overnight under vacuum.

25 1 equivalent of the dry product and methanol ($22.5\text{ml} \times \#g$ of starting material) was added to a round bottom flask equipped with a H_2O condenser and stirring bar. 1.2 equivalents of hydrazine mono hydrate was added and the mixture refluxed for 4 hours. After cooling to room temperature, concentrated HCl ($4.5\text{ml} \times \#g$ of starting material) was carefully added. Upon completion of the 30 addition, the mixture was refluxed overnight (> 8 hours). The reaction was cooled to 0°C and the precipitated by-product was removed by filtration. The filtrate was then concentrated in vacuo.

5 acid was then purified by reverse phase HPLC, verified by electrospray mass spectrometry and lyophilized to a powder.

10

EXAMPLE 2 Synthesis of compounds 1-15, 41, 43



5 (-)-2-oxo-4-thiazolidinecarboxylic acid; compound 39, 1-
cyclohexene-1-carboxylic acid; compound 40, (4R)-(-)-2-
thioxo-4-thiazolidinecarboxylic acid; compound 45,
10 cyclobutanecarboxylic acid; compound 46, cyclopentane-
carboxylic acid; compound 47, cyclohexanecarboxylic acid;
compound 48, 3,4-dihydro-2,2-dimethyl-4-oxo-2H-pyran-6-
carboxylic acid; compound 49, ethyl 1,3-dithiolane-2-
15 carboxylate (2 equivalents of the ethyl ester was
saponified with 3 equivalents of LiOH•H₂O in THF/H₂O (3/1)
The reaction was monitored by TLC (9/1 DCM/MeOH). Upon
completion, the mixture was acidified to pH 2 with 1M HCl
and then concentrated *in vacuo*. The resulting solid was
used without further purification); compound 50,
20 cyclopropanecarboxylic acid; compound 51, tetrahydro-2-
furoic acid), 2 equivalents of EDC, 1 equivalent of HObt
and 3 equivalents of DIPEA were dissolved DMA. The
reaction was stirred at room temperature and monitored by
TLC (9/1 DCM/MeOH). Upon completion, the mixture was
concentrated *in vacuo*. The resulting oil was re suspended
25 in Et₂O and washed twice with 0.1 N H₂SO₄, twice with
saturated NaHCO₃, and once with brine. The organic layer
was then dried over MgSO₄, filtered and concentrated *in
vacuo*. The residue was then purified on silica gel using
5% methanol in DCM as eluent to provide pure methyl
ester.

30 1 equivalent of the resultant methyl ester was dissolved
in THF/H₂O (3/1) and 3 equivalents of LiOH•H₂O was added.
The reaction was monitored by TLC (9/1 DCM/MeOH). Upon
completion, the mixture was acidified to pH 2 with 1M HCl
35 and then concentrated *in vacuo*. The resulting solid was
re suspended in Et₂O and washed twice with 0.1 M HCl and
once with brine. The organic layer was then dried over
MgSO₄, filtered and concentrated *in vacuo*. The resulting

5 2.3 equivalents of lithium iodide was added to 1 equivalent of the methyl ester in pyridine, and the mixture heated at reflux for 8 hours. The reaction was concentrated *in vacuo* and the residue was partitioned between EtOAc and 1N HCl. The aqueous layer was extracted
10 three times with EtOAc, and the combined organic layers were washed with 1M NaHCO₃, dried over MgSO₄ and concentrated *in vacuo*. The residue was dissolved in NMM and the solution concentrated *in vacuo*. The residue was taken up in DCM and then washed three times with 1N HCl.
15 The organic layer was dried over MgSO₄ and concentrated *in vacuo* to provide the benzoic acid in high enough purity to be used without further purification.

20 1 equivalent of the acid, 2 equivalents of commercially available β -Boc- diaminopropionic acid methyl ester, 2 equivalents of EDC, 1 equivalent of Hobt and 3 equivalents of DIPEA were dissolved DMA. The reaction was stirred at room temperature and monitored by TLC (9/1 DCM/MeOH). Upon completion, the mixture was concentrated
25 *in vacuo*. The resulting oil was re suspended in Et₂O and washed twice with 0.1 N H₂SO₄, twice with saturated NaHCO₃, and once with brine. The organic layer was then dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was then purified on silica gel using 5% methanol
30 in DCM as eluent to provide pure methyl ester.

35 The Boc protected amine was dissolved in a solution of TFA in DCM (1:1). After 20 minutes, the reaction was concentrated *in vacuo*. The resulting oil was dissolved in toluene and then reconcentrated *in vacuo*. 1 equivalent of this amine, 2 equivalents of the appropriate commercially available carboxylic acid (compound 16, N- acetyl-D-proline; compound 17, N- acetyl-L-proline; compound 38,

5 CO for 10 minutes. 0.15 equivalents palladium(II) acetate
and 0.15 equivalents of 1, 3- bis(diphenylphosphino)
propane were then added and the mixture was then degassed
while stirring with CO for another 10 minutes at which
time 2.5 equivalents of diisopropyl ethyl amine was
10 added. After properly assembling the bomb, it was charged
with 300psi CO gas and heated to 70°C with stirring
overnight. The bomb was then cooled and vented. The
mixture was transferred to a round bottom flask and
concentrated in vacuo. The residue was then purified on
15 silica gel using DCM with 1% acetone and 1% TEA as eluent
to provide pure methyl ester.

The Boc protected amine was dissolved in a solution of
TFA in DCM (1:1). After 20 minutes, the reaction was
20 concentrated in vacuo. The resulting oil was dissolved in
toluene and then reconcentrated in vacuo. The TFA salt of
the amine was dissolved in Et₂O and washed twice with a
10% solution of K₂CO₃ in H₂O and once with brine. The
organic layer was then dried over MgSO₄, filtered and
25 concentrated in vacuo.

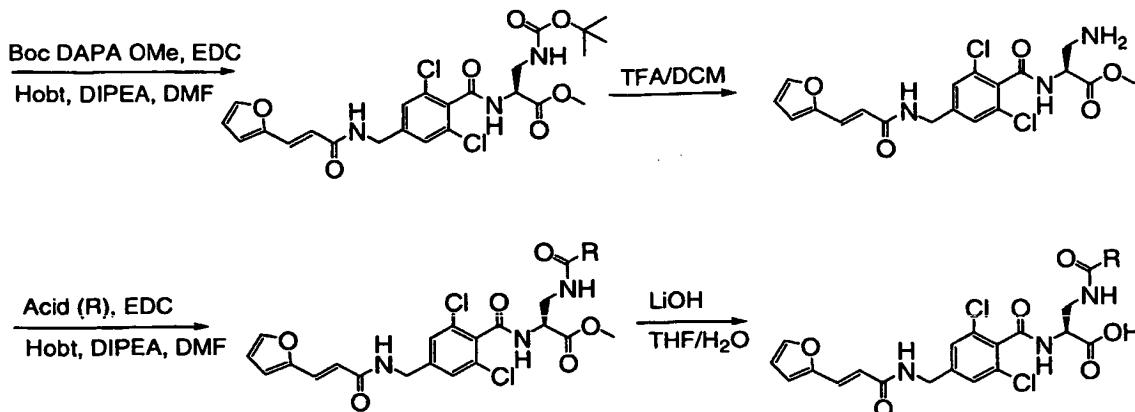
1 equivalent of the free based amine, 3 equivalents of
furylacrylic acid, 3 equivalents of EDC and 1 equivalent
of HObt were dissolved DMA. The reaction was stirred at
30 room temperature and monitored by TLC (9/1 DCM/MeOH).
Upon completion, the mixture was concentrated in vacuo.
The resulting oil was re suspended in Et₂O and washed
twice with 0.1 N H₂SO₄, twice with saturated NaHCO₃, and
once with brine. The organic layer was then dried over
35 MgSO₄, filtered and concentrated in vacuo. The residue was
then purified on silica gel using 5% methanol in DCM as
eluent to provide pure methyl ester.

5 The reaction was cooled to 0°C and the precipitated by-product was removed by filtration. The filtrate was then concentrated in vacuo.

10 The crude amine residue was dissolved in a 3:2 THF/H₂O solution. 1.1 equivalents of solid NaHCO₃ and 1.1 equivalents of Boc₂O were added and the mixture was stirred overnight. The reaction was concentrated, and the residue was partitioned between H₂O and Et₂O. The aqueous layer was extracted with Et₂O and the combined organic 15 layers were dried over MgSO₄ and concentrated in vacuo to a solid. Recrystallization from hot methanol and H₂O provided pure product.

20 1 equivalent of the Boc protected amine and 1.5 equivalents of 2, 6-lutidine was dissolved, with mild heating when necessary, in DCM in a round bottom flask. Once the starting material had completely dissolved, the mixture was cooled to -78°C under N₂ with a dry ice ethanol bath. Once cool, 2.5 equivalents of triflic 25 anhydride was added and the reaction was allowed to slowly come to room temperature with stirring. The reaction was monitored by TLC and was generally done in 4 hours. Upon completion, the reaction was concentrated in vacuo and the residue partitioned between EtOAc and H₂O. 30 The organic layer was washed twice with 0.1N H₂SO₄, twice with saturated NaHCO₃, once with brine, dried over MgSO₄ and concentrated in vacuo. The residue was then purified on silica gel using DCM as eluent to provide pure triflate.

35 1 equivalent of triflate was dissolved in DMF and MeOH in the glass insert of a high pressure Parr bomb. The starting material was then degassed while stirring with



A round bottom flask was equipped with an efficient overhead stirrer and charged with concentrated H₂SO₄ (2.7 x volume of H₂O) and H₂O and cooled to ~-5°C with an ethanol/ice bath. Once cool, 1 equivalent 2.6 dichloro phenol and 1 equivalent of N-(hydroxymethyl)phthalimide were added with vigorous stirring. The reaction was kept cool for 4 hours and then allowed to warm to room temperature overnight with constant stirring. The reaction generally proceeded to a point where there was just a solid in the round bottom flask. At that point EtOAc and H₂O were added and stirred into the solid. Large chunks were broken up and then the precipitate was filtered and washed with more EtOAc and H₂O. The product was then used without further purification after drying overnight under vacuum.

1 equivalent of the dry product and methanol (22.5ml x #g of starting material) was added to a round bottom flask equipped with a H₂O condenser and stirring bar. 1.2 equivalents of hydrazine mono hydrate was added and the mixture refluxed for 4 hours. After cooling to room temperature, concentrated HCl (4.5ml x #g of starting material) was carefully added. Upon completion of the addition, the mixture was refluxed overnight (> 8 hours).

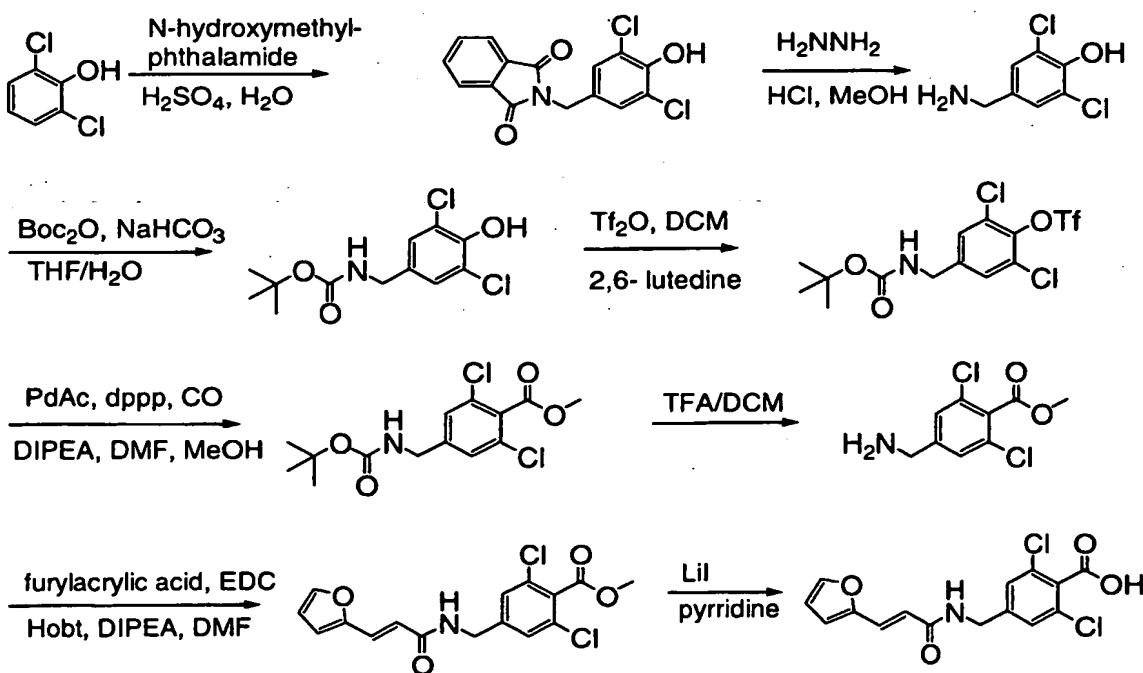
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EXAMPLES

Abbreviations used in the following section: Boc = t-butyloxycarbonyl; Boc₂O = t-butyloxycarbonyl anhydride; DMA = dimethylacetamide; DMF = dimethylformamide; HObt = 1-hydroxybenztriazole; TFA = trifluoroacetic acid; DCM = dichloromethane; MeOH = methanol; HOAc = acetic acid; HCl = hydrochloric acid; H₂SO₄ = sulfuric acid; K₂CO₃ = potassium carbonate; THF = tetrahydrofuran; EtOAc = ethyl acetate; DIPEA = diisopropylethylamine; NaHCO₃ = sodium bicarbonate; ACN = acetonitrile; Na₂•EDTA = ethylenediaminetetraacetic acid sodium salt; TBAF = tetrabutyl ammonium fluoride; EDC = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide•HCl; TEA = triethylamine; MgSO₄ = magnesium sulfate; TES = triethylsilane; Et₂O = diethyl ether; BBr₃ = boron tribromide

EXAMPLE 1 Synthesis of compounds 16, 17, 38-40, 46-50

25



5 phosphate); lubricants (e.g., magnesium stearate, talc or silica); disintegrates (e.g., starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulfate). Tablets may be coated by methods well known in
10 the art. The preparations may also contain flavoring, coloring and/or sweetening agents as appropriate.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing
15 predetermined amounts of the active ingredients; as powders or granules; as solutions or suspensions in an aqueous liquid or a non-aqueous liquid; or as oil-in-water emulsions or water-in-oil liquid emulsions. A tablet may be made by compression or molding,
20 optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine, the active ingredients in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent,
25 preservative, surface active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or
30 controlled release of the active ingredients therein.

5 effect delivering the compounds to the alimentary canal for exposure to the mucosa thereof. Accordingly, the formulation can consist of material effective in protecting the compound from pH extremes of the stomach, or in releasing the compound over time, to optimize the
10 delivery thereof to a particular mucosal site. Enteric coatings for acid-resistant tablets, capsules and caplets are known in the art and typically include acetate phthalate, propylene glycol and sorbitan monoleate.

15 Various methods for producing formulations for alimentary delivery are well known in the art. See, generally *Remington's Pharmaceutical Sciences*, 18th Ed., Gennaro, ed., Mack Publishing Co., Easton, PA, 1990. The formulations of the invention can be converted in a known manner into the customary formulations, such as tablets, coated tablets, pills, granules, aerosols, syrups, emulsions, suspensions and solutions, using inert, non-toxic, pharmaceutically suitable excipients or solvents. The therapeutically active compound should in each case be present in a concentration of about 0.1% to about 99% by weight of the total mixture, that is to say in amounts which are sufficient to achieve the desired dosage range. The formulations are prepared, for example, by extending the active compounds with solvents and/or excipients, if appropriate using emulsifying agents and/or dispersing agents, and, for example, in the case where water is used as the diluent, organic solvents can be used as auxiliary solvents if appropriate.

35 Compositions may also be formulated with binding agents (e.g., pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose or calcium hydrogen

5 and creams and aerosols for inhalation. Formulations for non-parenteral administration may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives. Pharmaceutically acceptable organic or inorganic carrier substances suitable for non-parenteral administration which do not deleteriously react with compounds of the invention can be used. Suitable pharmaceutically acceptable carriers include, but are not limited to, water, salt solutions, alcohol, polyethylene glycols, gelatin, lactose, amylose, 10 magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose, polyvinylpyrrolidone and the like. The formulations can be sterilized and, if desired, mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, 15 salts for influencing osmotic pressure, buffers, colorings flavorings and/or aromatic substances and the like which do not deleteriously react with compounds of the invention. Aqueous suspensions may contain substances which increase the viscosity of the suspension 20 including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. Optionally, the suspension may 25 also contain stabilizers.

Compounds of the invention exhibit high oral 30 bioavailability. Accordingly, in a preferred embodiment, compounds of the invention are administered via oral delivery. Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets, troches, tablets or 35 SECs (soft elastic capsules or caplets). Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids, carrier substances or binders may be desirably added to such formulations. Such formulations may be used to

5 The actual amount of compound administered and the route
of administration will depend upon the particular disease
or condition as well as other factors such as the size,
age, sex and ethnic origin of the individual being
treated and is determined by routine analysis. In
10 general, intravenous doses will be in the range from
about 0.01-1000 mg/kg of patient body weight per day,
preferably 0.1 to 20 mg/kg and more preferably 0.3 to 15
mg/kg. Administration may be once or multiple times per
day for several days, weeks or years or may be a few
15 times per week for several weeks or years. The amount of
compound administered by other routes will be that which
provides a similar amount of compound in plasma compared
to the intravenous amounts described which will take into
consideration the plasma bioavailability of the
20 particular compound administered.

In methods of the invention, the compound may be
administered orally (including buccal, sublingual,
inhalation), nasally, rectally, vaginally, intravenously
25 (including intra-arterially), intradermally,
subcutaneously, intramuscularly and topically. Compounds
will be formulated into compositions suitable for
administration for example with carriers, diluents,
thickeners, adjuvants etc. as are routine in the
30 formulation art. Accordingly, another aspect of the
invention provides pharmaceutical compositions comprising
a compound of formula (I) and a pharmaceutically
acceptable carrier, excipient or adjuvant and may also
include additional active ingredients such as anti-
35 inflammatories e.g. NSAIDs.

Dosage forms include solutions, powders, tablets,
capsules, gel capsules, suppositories, topical ointments

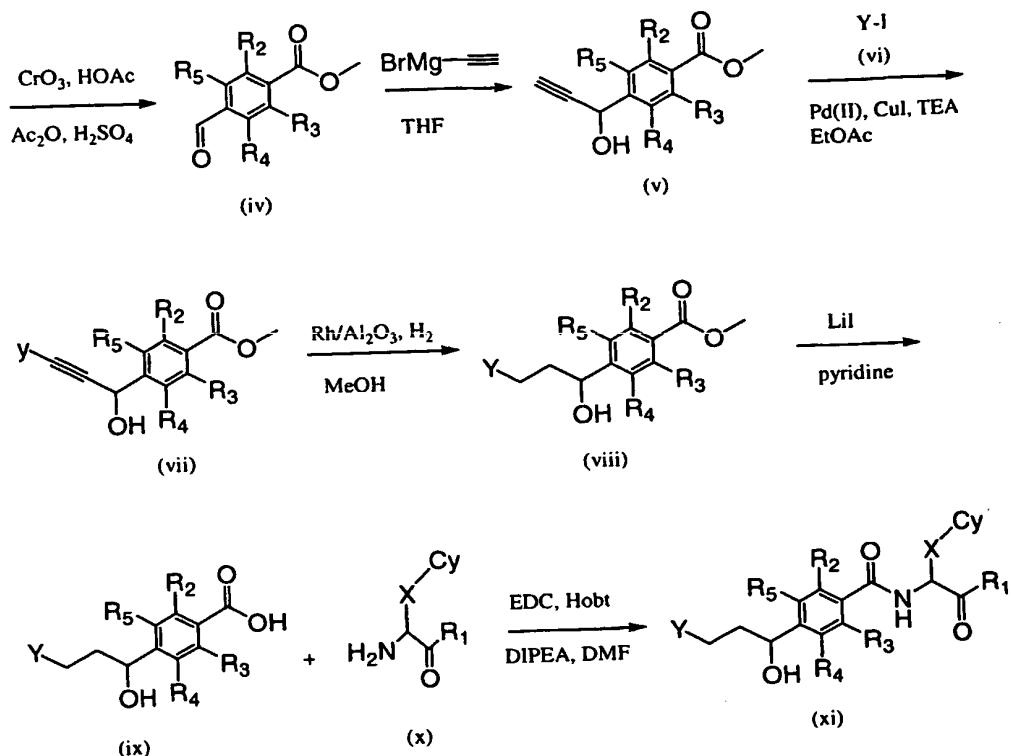
5 Compounds of the invention or compositions thereof are useful in treating conditions or diseases including: psoriasis; responses associated with inflammatory bowel disease (such as Crohn's disease and ulcerative colitis), dermatitis, meningitis, encephalitis, uveitis, allergic
10 conditions such as eczema and asthma, conditions involving infiltration of T-cells and chronic inflammatory responses, skin hypersensitivity reactions (including poison ivy and poison oak); atherosclerosis, autoimmune diseases such as rheumatoid arthritis,
15 systemic lupus erythematosis (SLE), diabetes mellitus, multiple sclerosis, Reynaud's syndrome, autoimmune thyroiditis, experimental autoimmune encephalomyelitis, Sjorgen's syndrome, juvenile onset diabetes, and immune responses associated with delayed hypersensitivity
20 mediated by cytokines and T-lymphocytes typically found in tuberculosis, sarcoidosis, polymyositis, granulomatosis and vasculitis; pernicious anemia; diseases involving leukocyte diapedesis; CNS inflammatory disorder, multiple organ injury syndrome secondary to
25 septicaemia or trauma; autoimmune hemolytic anemia; myasthenia gravis; antigen-antibody complex mediated diseases; all types of transplantations, including graft vs. host or host vs. graft disease, HIV and rhinovirus infection, pulmonary fibrosis, alopecia, scleredoma,
30 endometriosis, vitiligo, ischemic reperfusion injury mediated by neutrophils such as acute myocardial infarction, restenosis following PTCA, invasive procedures such as cardiopulmonary bypass surgery, cerebral edema, stroke, traumatic brain injury,
35 hemorrhagic shock, burns, ischemic kidney disease, multi-organ failure, wound healing and scar formation, atherosclerosis.

alkyl or alkoxy. In more preferred embodiments, Y is 3-hydroxy-phenyl or 3-chloro-phenyl.

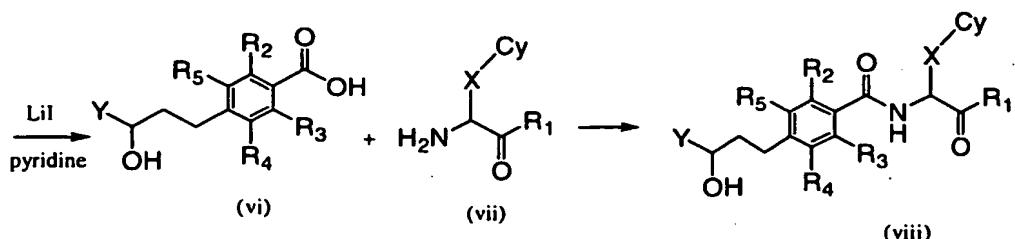
Compounds of the invention bind to LFA-1 preferentially over Mac-1. Accordingly, in an aspect of the invention, there is provided a method of inhibiting the binding of LFA-1 to ICAMs (cellular adhesion molecules), the method comprising contacting LFA-1 with a compound of formula (I). The method may be carried out in vivo or ex vivo as a solution based or cell based assay wherein the compound of the invention is introduced to LFA-1 in the presence of a putative or known ligand (such as ICAM-1). The compound of the invention may be labeled, for example isotopically radiolabeled, or labeled with a fluorophore such as fluorescein isothiocyanate (FITC), to facilitate detection of ligand binding or reduction thereof to the protease. Thus compounds of the invention are useful for diagnostic and screening assays.

5

Compounds of the invention are therapeutically and/or prophylactically useful for treating diseases or conditions mediated by LFA-1 activity. Accordingly in an aspect of the invention, there is provided a method of 10 treating a disease or condition mediated by LFA-1 in a mammal, i.e. a human, comprising administering to said mammal an effective amount of a compound of the invention. By "effective amount" is meant an amount of compound which upon administration is capable of reducing 15 the activity of LFA-1; or the amount of compound required to prevent, inhibit or reduce the severity of any symptom associated with an LFA-1 mediated condition or disease upon administration.



Referring to scheme 5, starting compound (i) is reacted with triflic anhydride and 2,6-lutidine to give intermediate (ii) which is converted to methyl ester (iii) by reacting with palladium(II)acetate, 1,3-bis(diphenylphosphino)propane (dppp) and subsequently with diisopropyl ethylamine (DIPEA) in DMF and methanol. The ester (iii) is then reacted with CrO_3 in acetic acid and anhydride to give aldehyde (iv) which is reacted with Grignard reagent ethynyl-magnesium bromide in THF to give alkyne intermediate (v). Iodo reagent (vi) Y-I is reacted with (v) to give intermediate (vii) which is converted to the alkane (viii) by reacting with $\text{Rh}/\text{Al}_2\text{O}_3$ under H_2 atmosphere. The methyl ester is converted to free acid (ix) with LiI in pyridine which is then coupled to amino acid residue (x) to give compound of the invention (xi). In preferred embodiments of scheme 5, Y is phenyl, optionally substituted with hydroxy, halogen,

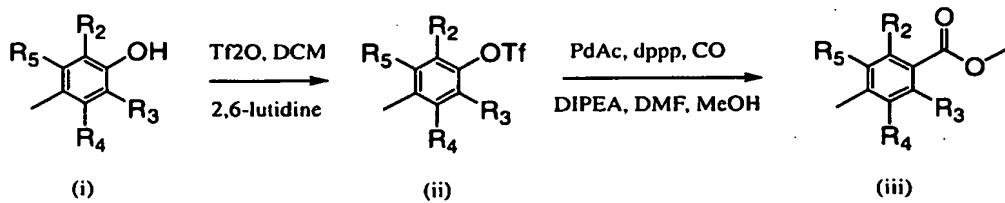


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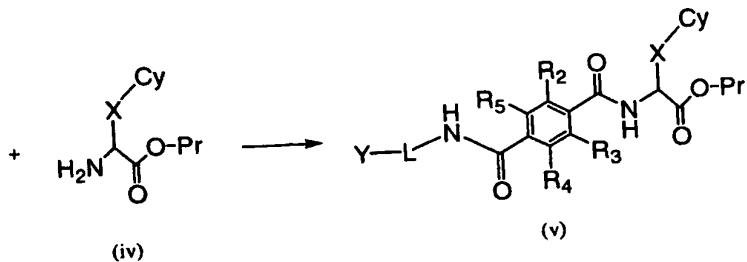
Referring to scheme 4, starting compound (i), prepared according to the procedures described in scheme 2, is converted to the iodo intermediate (ii) and reacted with alkyne (iii) to give intermediate (iv). Alkyne (iii) is prepared by reacting Y-COOH with Br-C≡CH in THF. Intermediate (iv) is then converted to the alkane (v) by reacting with Rh/Al₂O₃ in H₂ atmosphere and the ester group converted to the free acid by reacting with LiI in pyridine to give (vi). Intermediate (vi) is reacted with amino acid (vii) to give compound of the invention (viii). In a particular embodiment of scheme 4, Y is phenyl optionally substituted with alkyl, hydroxy or halogen. In a particularly preferred embodiment Y is 3-chloro-phenyl or 3-hydroxy-phenyl.

In another particular embodiment, compounds of formula (Ie) of the invention may be prepared according to scheme 5.

Scheme 5



WO 02/059114



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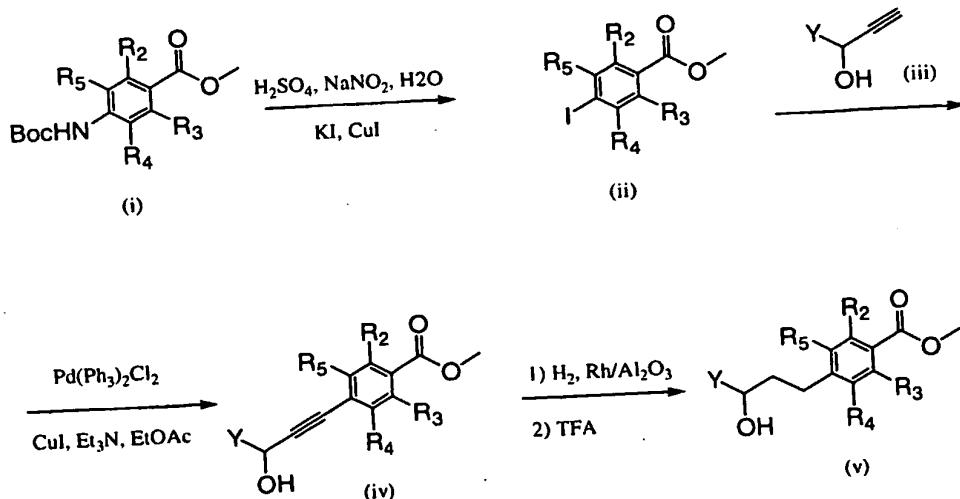
Referring to scheme 3, carboxylate starting reagent (i) is coupled with amine reagent (ii) $Y-L-NHR_6$ to give intermediate (iii) which is coupled with (iv) to yield compound of the invention (v). In a preferred embodiment of scheme 3, $Y-L-$ is benzyl, optionally substituted with hydroxy, halogen, alkyl or alkoxy. More preferably $Y-L-$ is 3-hydroxy-benzyl.

15

In another particular embodiment, compounds of formula (Id) of the invention may be prepared according to scheme 4.

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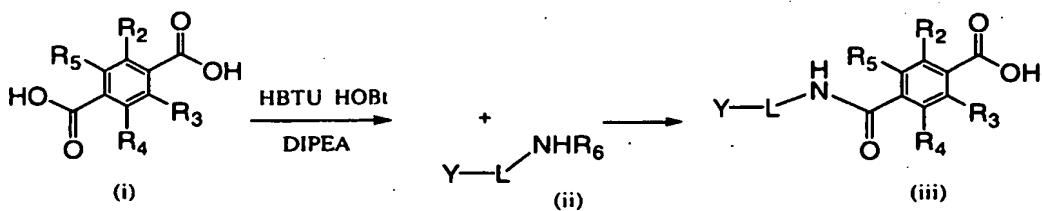
Scheme 4



5 Referring to scheme 2, starting compound (i), commercially available or synthesized from commercially available reagents, is reacted with N-hydroxymethylphthalimide to give intermediate (ii) which is reacted with hydrazine to yield the free amine (iii).
 10 The amine is Boc protected (iv) by reacting with Boc_2O and sodium bicarbonate and then reacted with triflic anhydride to give intermediate (v). The triflate intermediate (v) is then converted to the methyl ester intermediate (vi) by reacting with palladium(II) acetate and 1,3-
 15 bi(diphenylphosphino)propane (dpdp) and subsequently with diisopropyl ethylamine (DIPEA). The Boc group of (vi) is removed with TFA and then reacted with carboxylic acid (vii) to give intermediate (viii). In a preferred embodiment of scheme 2, intermediate (vii) Y-L-C(O)OH is
 20 furylacrylic acid or thienylacrylic acid. The methyl ester of (viii) is removed with LiOH to give the free acid which is reacted with the N-Boc protected diaminopropanoic acid/ester (x) to yield intermediate (xi). The Boc group of (xi) is removed with TFA and then reacted with carboxyl-substituted non-aromatic ring (xii)
 25 to give final compound (Ib) of the invention.

In another particular embodiment compounds of formula (Ic) of the invention may be prepared according to scheme 30 3.

Scheme 3

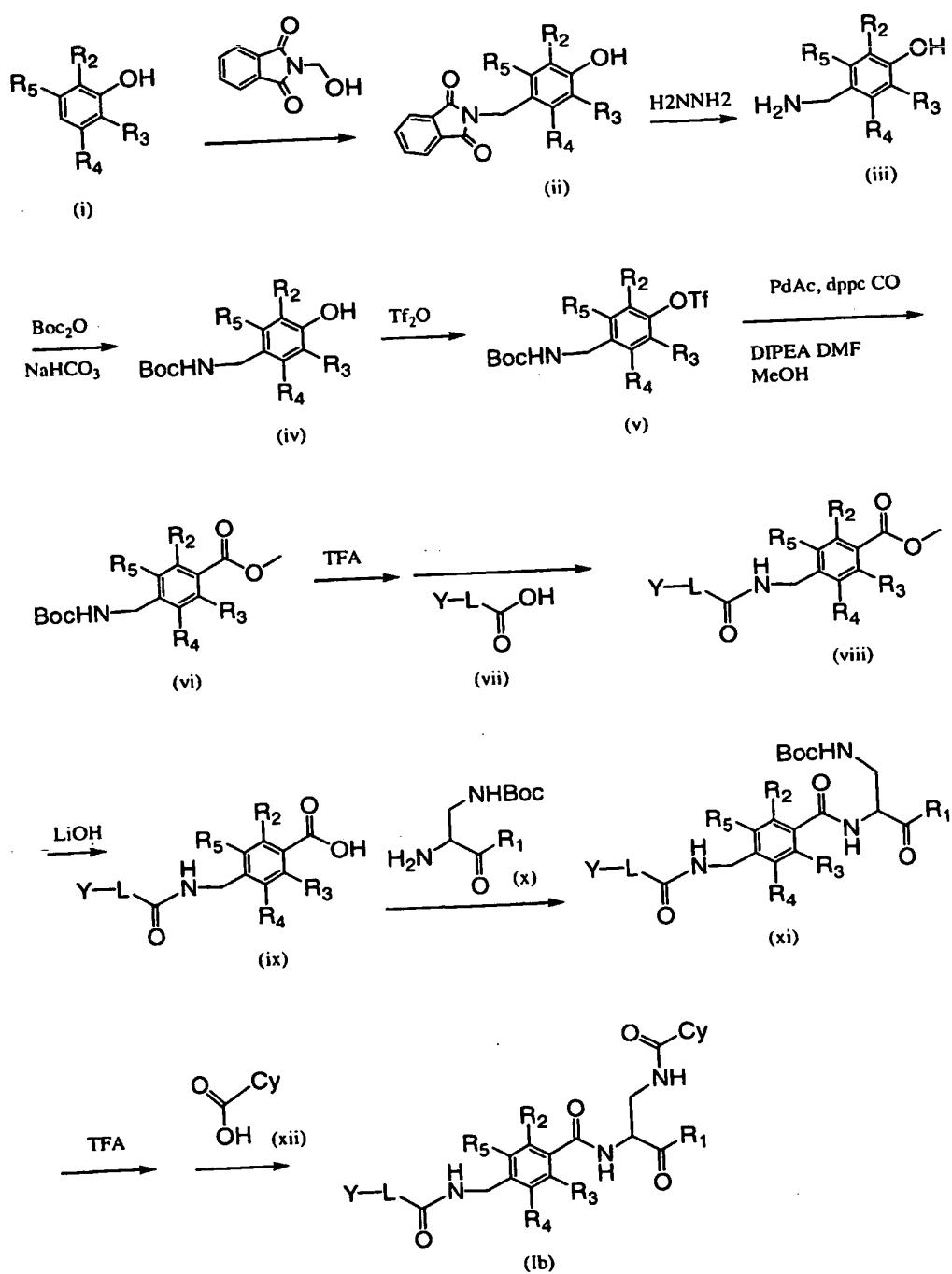


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In a particular embodiment, compounds of formula (Ib) of the invention may be prepared according to scheme 2.

Scheme 2

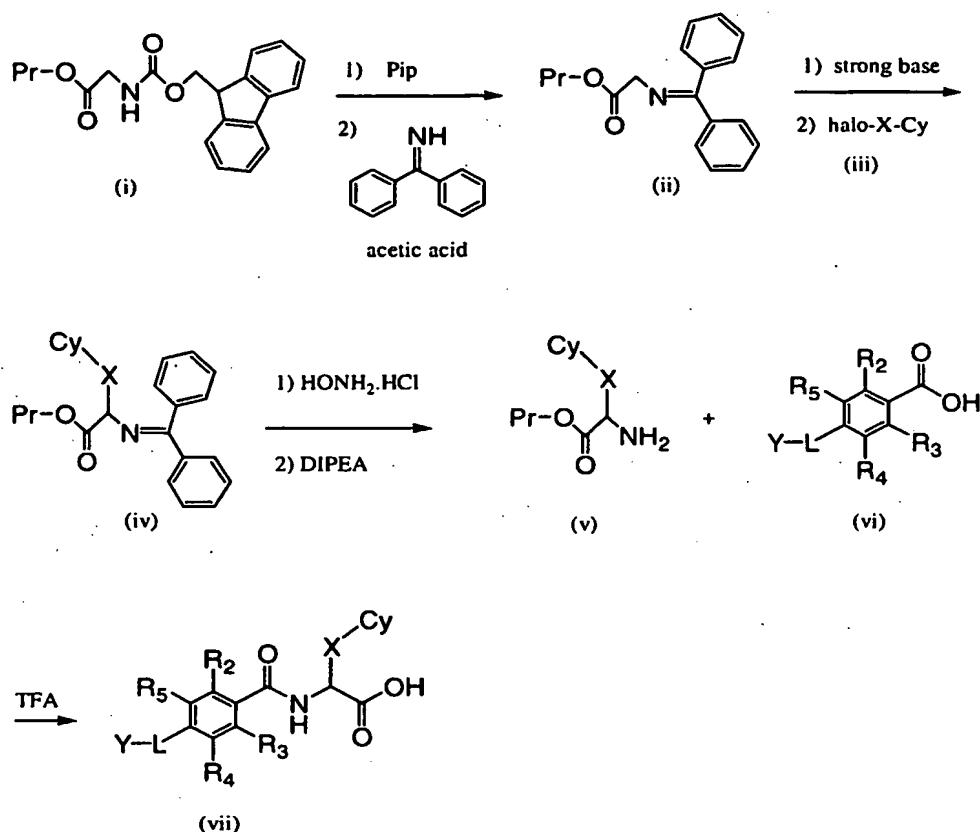
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Scheme 1



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Referring to scheme 1, a commercially available glycine amino acid residue is protected at the amino (e.g. fmoc) and carboxyl groups (Pr) or else immobilized on a solid support. The amino protecting group is removed with a suitable reagent and is reacted with diphenylketimine and subsequently alkylated at the alpha carbon with (iii) halo-X-Cy to give intermediate (vi). The imine (vi) is converted to the free amine (v) and then coupled with intermediate (vi) to provide the compound of the invention which is optionally deprotected at the carboxyl group to give free acid (vii). The free acid in turn may be esterified or amidated according to the definitions of substituent R₁.

5 as well as detailed protection and deprotection procedures. For example, suitable amino protecting groups include t-butyloxycarbonyl (Boc), fluorenyl-methyloxycarbonyl (Fmoc), 2-trimethylsilyl-ethyoxy-carbonyl (Teoc), 1-methyl-1-(4-biphenyl)ethoxycarbonyl (Bpoc), allyloxycarbonyl (Alloc), and benzyloxycarbonyl (Cbz). Carboxyl groups can be protected as fluorenyl-methyl groups, or alkyl esters i.e. methyl or ethyl, or alkenyl esters such as allyl. Hydroxyl groups may be protected with trityl, monomethoxytrityl, dimethoxytrityl, and trimethoxytrityl groups.

Compounds may be prepared according to organic synthetic procedures described in United States patent application 09/6446,330 filed on 14 September 2000, the entirety of which is incorporated herein by reference. Generally, compounds may be prepared according to reaction scheme 1.

5 pharmaceutically acceptable organic nontoxic bases includes salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as isopropylamine, trimethylamine,
10 diethylamine, triethylamine, tripropylamine, ethanolamine, 2-diethylaminoethanol, trimethamine, dicyclohexylamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, methylglucamine,
15 theobromine, purines, piperazine, piperidine, N-ethylpiperidine, polyamine resins and the like. Particularly preferred organic non-toxic bases are isopropylamine, diethylamine, ethanolamine, trimethamine, dicyclohexylamine, choline, and caffeine.

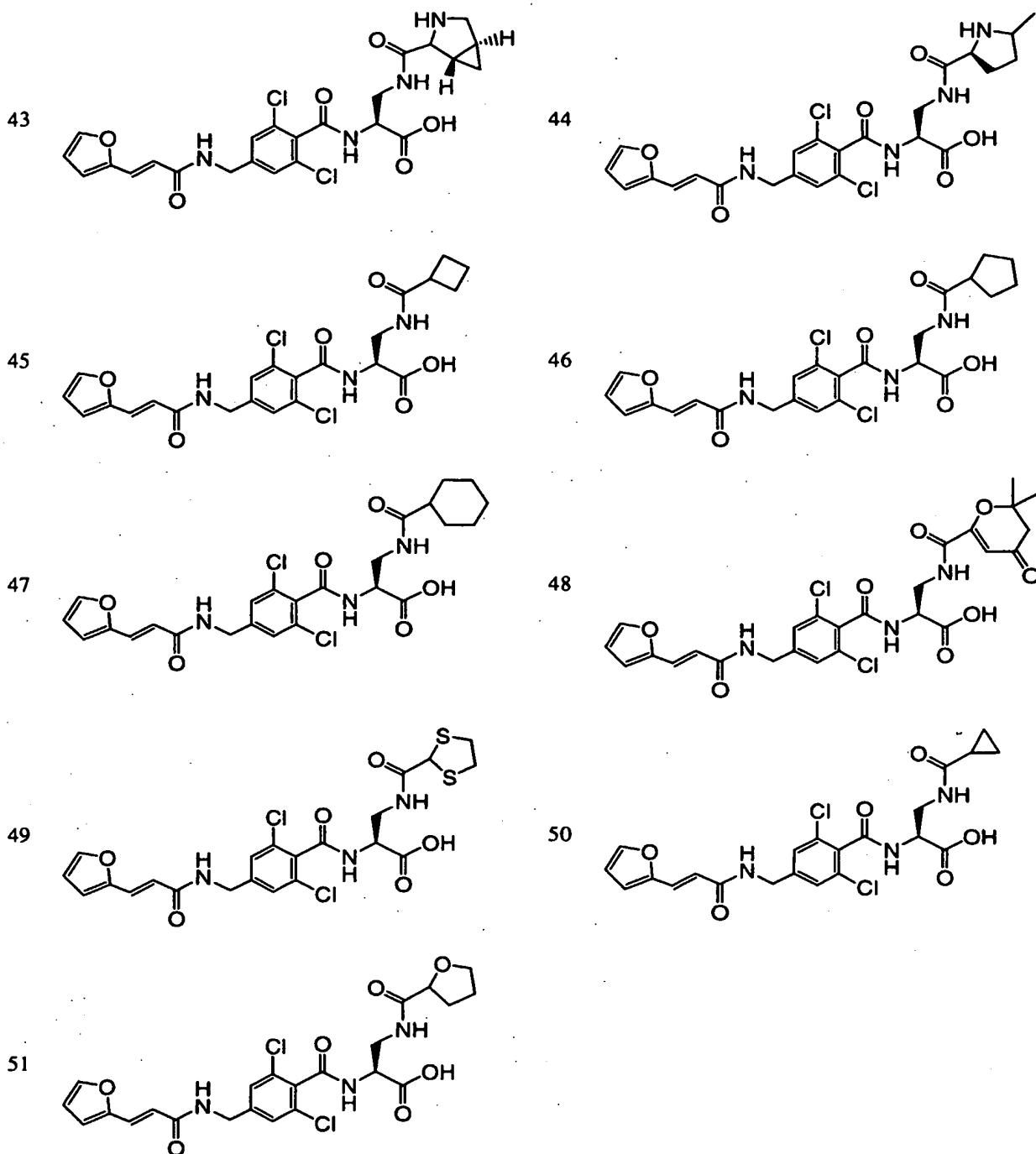
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Compounds of the invention may be prepared according to established organic synthesis techniques from starting materials and reagents that are commercially available or
25 from starting materials that may be prepared from commercially available starting materials. Many standard chemical techniques and procedures are described in March, J., "Advanced Organic Chemistry" McGraw-Hill, New York, 1977; and Collman, J., "Principles and Applications
30 of Organotransition Metal Chemistry" University Science, Mill Valley, 1987; and Larock, R., "Comprehensive Organic Transformations" Verlag, New York, 1989. It will be appreciated that depending on the particular substituents present on the compounds, suitable protection and
35 deprotection procedures will be required in addition to those steps described herein. Numerous protecting groups are described in Greene and Wuts, Protective Groups in Organic Chemistry, 2d edition, John Wiley and Sons, 1991,

5 contemplated and are within the scope of the invention whether in pure isomeric form or in mixtures of such isomers as well as racemates. Stereoisomeric compounds may be separated by established techniques in the art such as chromatography, i.e. chiral HPLC, or
10 crystallization methods.

"Pharmaceutically acceptable" salts include both acid and base addition salts. Pharmaceutically acceptable acid addition salt refers to those salts which retain the biological effectiveness and properties of the free bases and which are not biologically or otherwise undesirable, formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, carbonic acid, phosphoric acid and the like, and organic acids may be selected from aliphatic, cycloaliphatic, aromatic, arylaliphatic, heterocyclic, carboxylic, and sulfonic classes of organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, gluconic acid, lactic acid, pyruvic acid, oxalic acid, malic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, aspartic acid, ascorbic acid, glutamic acid, anthranilic acid, benzoic acid, cinnamic acid, mandelic acid, embonic acid, phenylacetic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid and the like.

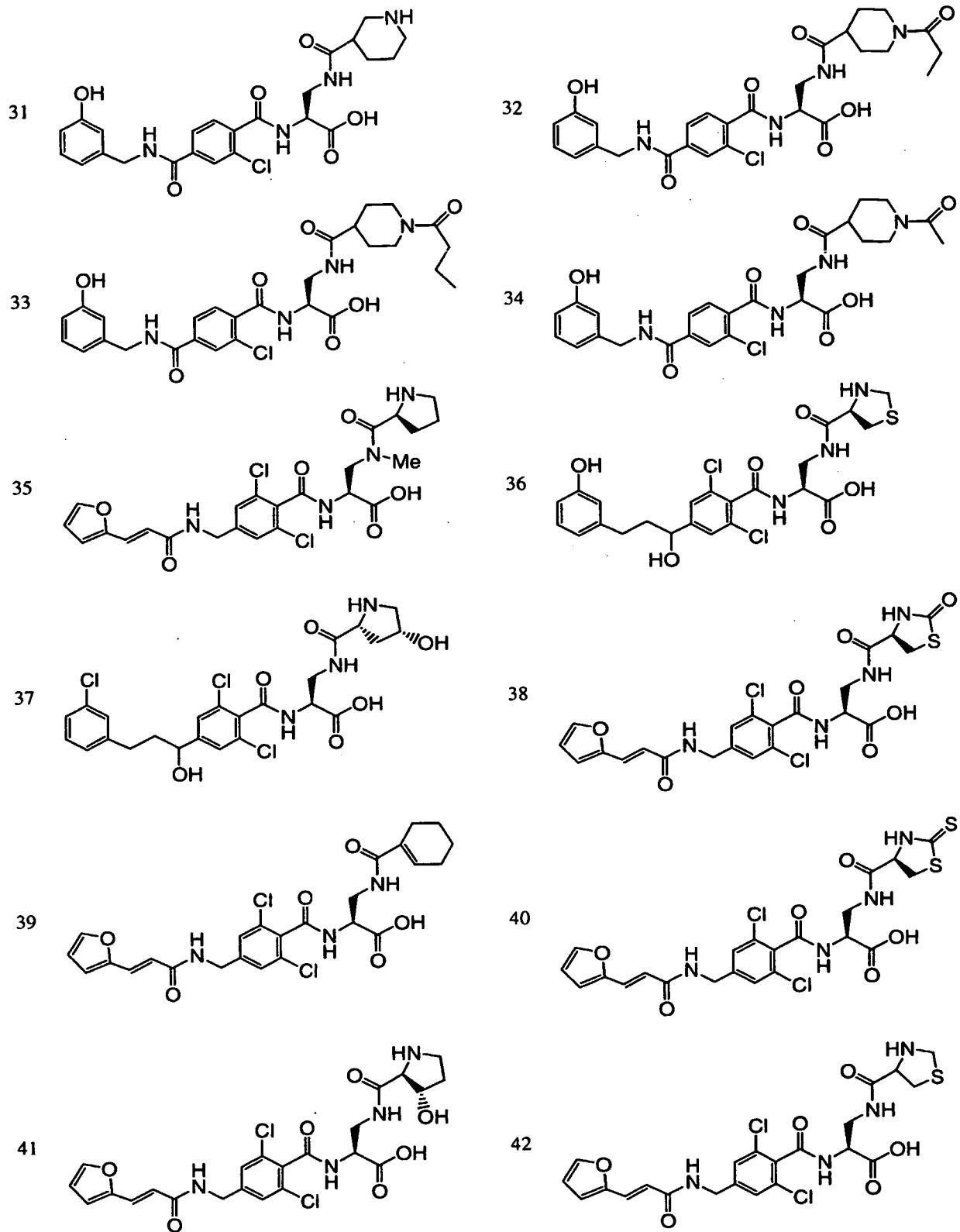
Pharmaceutically acceptable base addition salts include those derived from inorganic bases such as sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, aluminum salts and the like. Particularly preferred are the ammonium, potassium, sodium, calcium and magnesium salts. Salts derived from

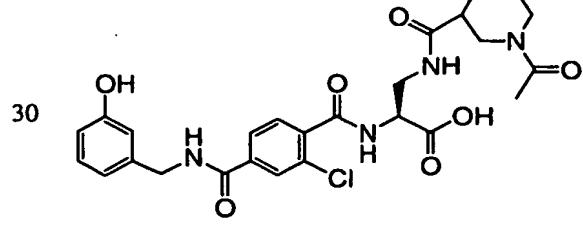
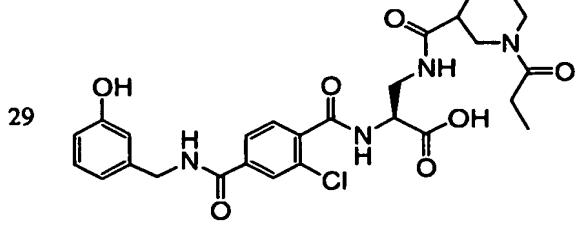
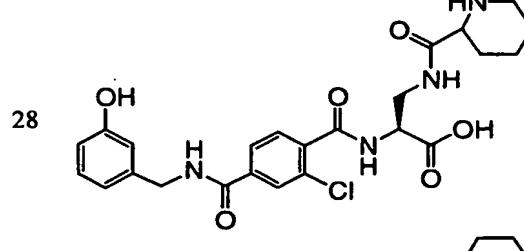
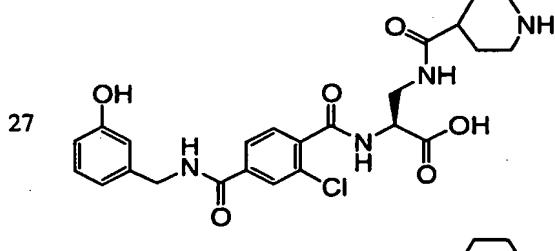
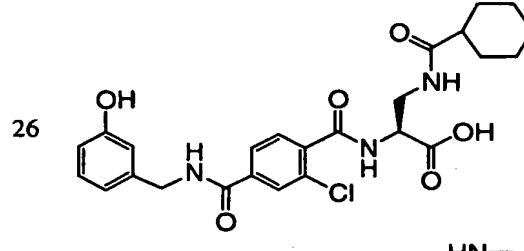
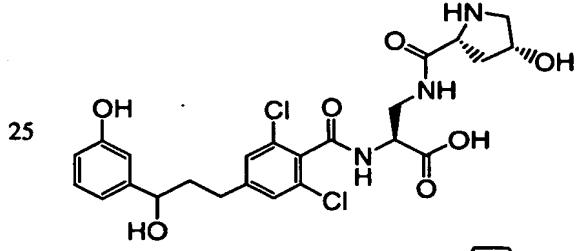
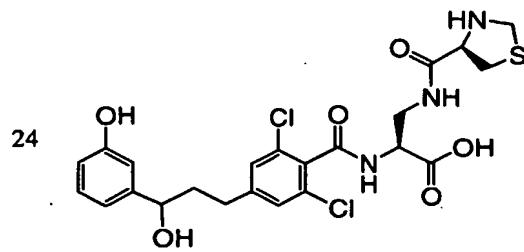
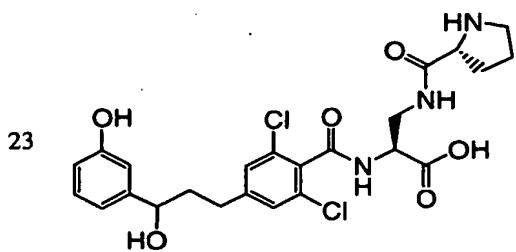
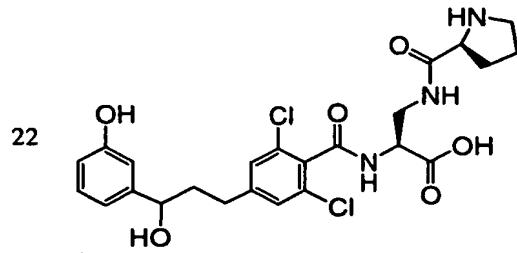
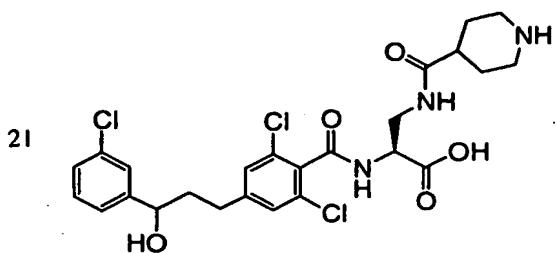
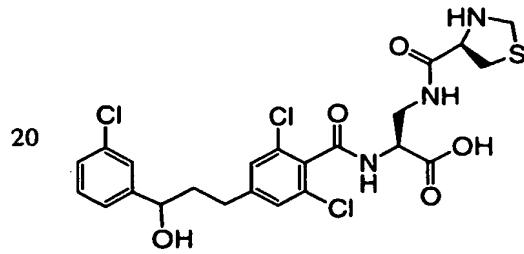
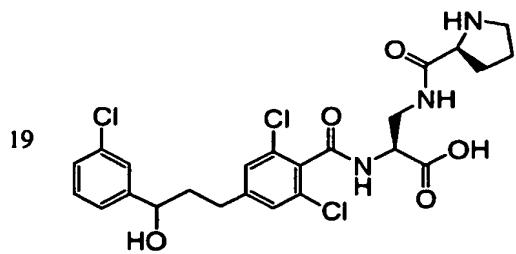


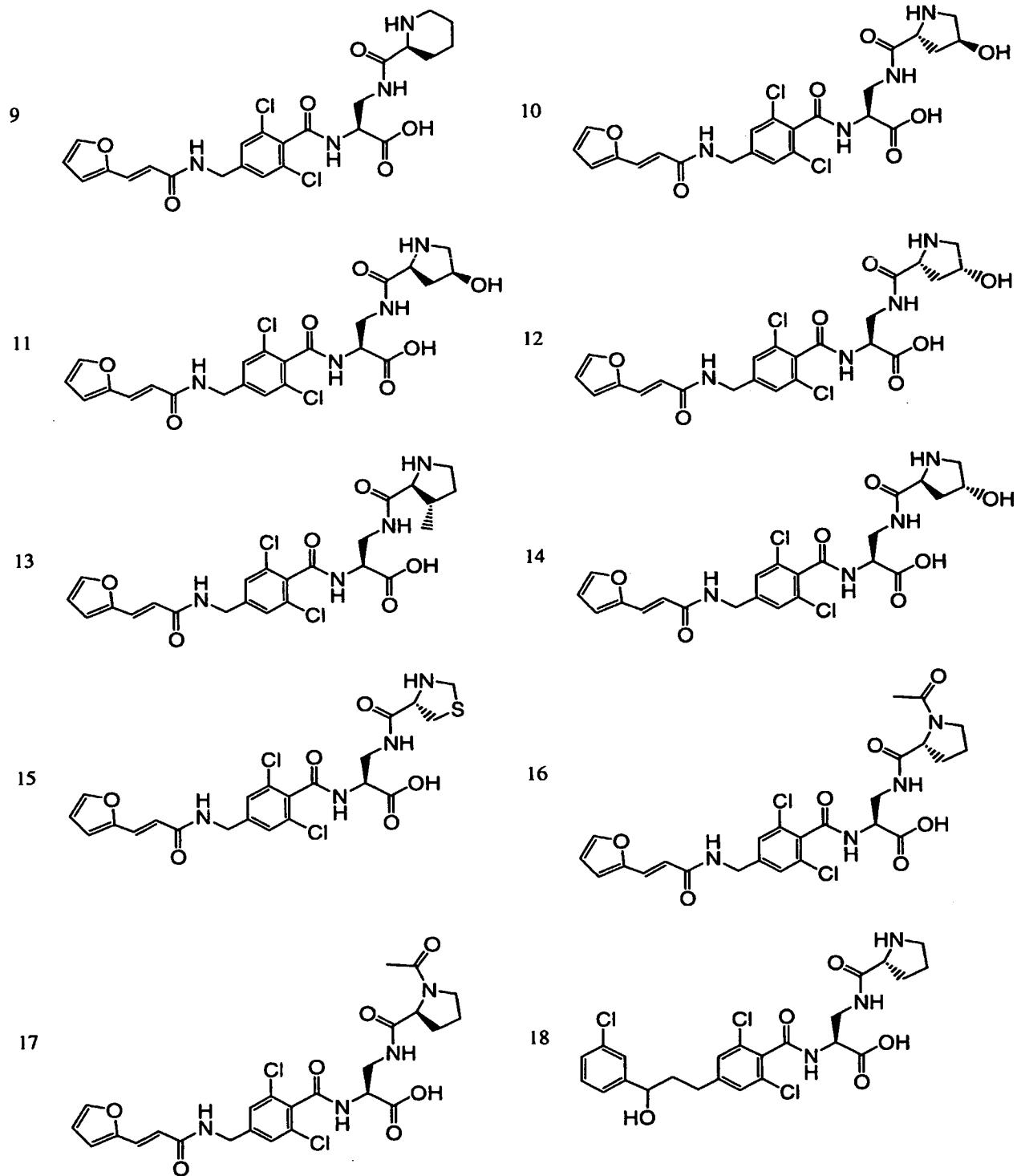
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and salts, solvates, hydrates and esters thereof.

10 It will be appreciated that compounds of the invention may incorporate chiral centers and therefore exist as geometric and stereoisomers. All such isomers are



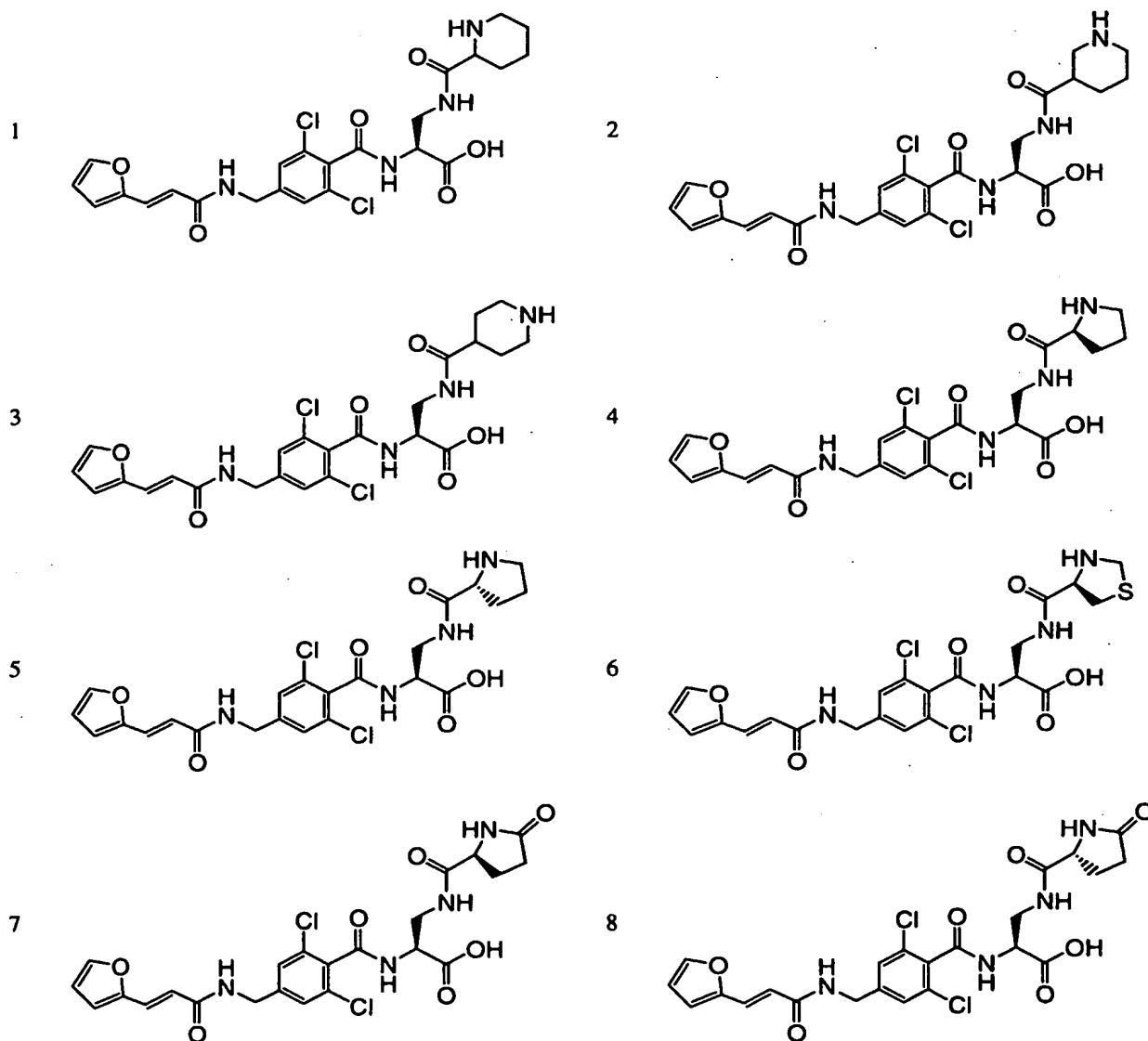


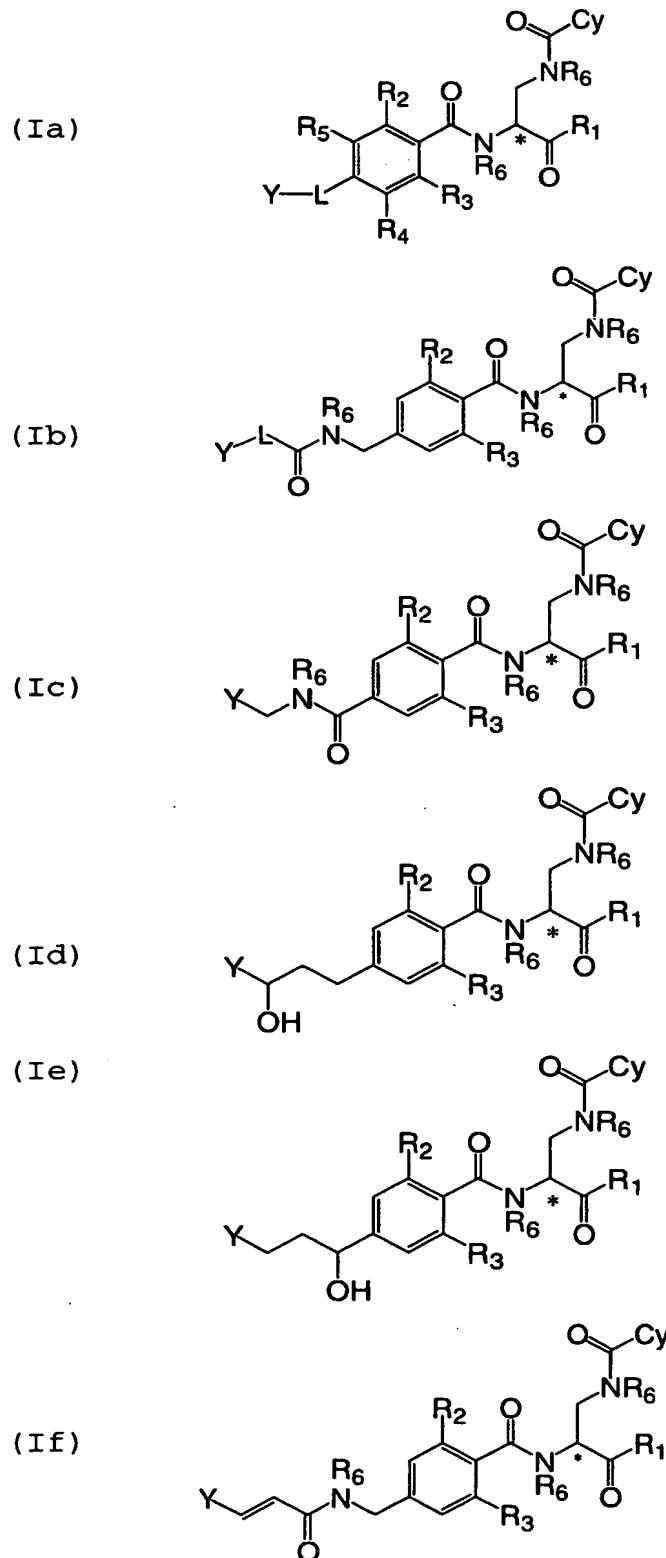


5 wherein Cy, Y, L and R₁₋₆ are as previously defined. In a particularly preferred embodiment, the carbon atom marked with an asterisk (*) in compounds of formula (Ia) - (If) is chiral. In a particular embodiment, the carbon atom has an R-configuration. In another particular embodiment, the carbon atom has an S-configuration.

10

Particular compounds of the invention include:





5 NH₂. In a particularly preferred embodiment R₁ is ethoxy.
In another particularly preferred embodiment R₁ is
isobutyloxy. In another particularly preferred
embodiment R₁ is alkoxy substituted with amino, for
example 2-aminoethoxy, N-morpholinoethoxy, N,N-
10 dialkyaminoethoxy, quaternary ammonium hydroxy alkoxy
(e.g. trimethylammoniumhydroxyethoxy).

R₂₋₅ are independently H, hydroxyl, mercapto, halogen,
15 cyano, amino, amidine, guanidine, nitro or alkoxy; or R₃
and R₄ together form a fused carbocycle or heterocycle
optionally substituted with hydroxyl, halogen, oxo, thio,
amino, amidine, guanidine or alkoxy. In a particular
embodiment R₂ and R₃ are independently H, F, Cl, Br or I.
In another particular embodiment, R₄ and R₅ are both H.
20 In another particular embodiment, one of R₂ and R₃ is a
halogen while the other is hydrogen or a halogen. In a
particularly preferred embodiment, R₃ is Cl while R₂, R₄
and R₅ are each H. In another particularly preferred
embodiment, R₂ and R₃ are both Cl while R₄ and R₅ are both
25 H.

R₆ is H or a hydrocarbon chain optionally substituted with
a carbocycle or a heterocycle. In a preferred
embodiment, R₆ is H or alkyl i.e. methyl, ethyl, propyl,
30 butyl, i-butyl, s-butyl or t-butyl. In a particular
embodiment R₆ is H.

In a preferred embodiment, compounds of the invention
have the general formula (Ia) - (If)

5 2-yl, phenyl substituted with a halogen (preferably Cl) or hydroxyl, preferably at the meta position.

L is a divalent hydrocarbon optionally having one or more carbon atoms replaced with N, O, S, SO or SO₂ and 10 optionally being substituted with hydroxyl, halogen oxo, or thio; or three carbon atoms of the hydrocarbon are replaced with an amino acid residue. Preferably L is less than 10 atoms in length and more preferably 5 or less and most preferably 5 or 3 atoms in length. In 15 particular embodiments, L is selected from the group consisting of -CH=CH-C(O)-NR₆-CH₂-, -CH₂-NR₆-C(O)-, -C(O)-NR₆-CH₂-, -CH(OH)-(CH₂)₂-, -(CH₂)₂-CH(OH)-, -(CH₂)₃-, -C(O)-NR₆-CH(R₇)-C(O)-NR₆-, -NR₆-C(O)-CH(R₇)-NR₆-C(O)-, -CH(OH)-CH₂-O- and -CH(OH)-CF₂-CH₂- wherein each R₆ is 20 independently H or alkyl and R₇ is an amino acid side chain. Preferred amino acid side chains include non-naturally occurring side chains such as phenyl or naturally occurring side chains. Preferred side chains are those from Phe, Tyr, Ala, Gln and Asn. In a 25 preferred embodiment L is -CH=CH-C(O)-NR₆-CH₂- wherein the -CH=CH- moiety thereof is adjacent (i.e. covalently bound) to Y. In another preferred embodiment, L is -CH₂-NR₆-C(O)- wherein the methylene moiety (-CH₂-) thereof is adjacent to Y.

30 R₁ is H, OH, amino, O-carbocycle or alkoxy optionally substituted with amino, a carbocycle or a heterocycle. In a preferred embodiment, R₁ is H, phenyl or C₁₋₄ alkoxy optionally substituted with a carbocycle such as phenyl. 35 In a particular embodiment R₁ is H. In another particular embodiment R₁ is methoxy, ethoxy, propyloxy, butyloxy, isobutyloxy, s-butyloxy, t-butyloxy, phenoxy or benzyloxy. In yet another particular embodiment R₁ is

5

In another preferred embodiment Cy is a 3-6 member carbocycle optionally substituted with hydroxyl, mercapto, halogen, oxo, thio, amino, amidine, guanidine, alkyl, alkoxy or acyl. In a particular embodiment the carbocycle is saturated or partially unsaturated. In particular embodiments Cy is a carbocycle selected from the group consisting of cyclopropyl, cyclopropenyl, cyclobutyl, cyclobutenyl, cyclopentyl, cyclopentenyl, cyclohexyl and cyclohexenyl.

15

X is a C₁₋₅ divalent hydrocarbon linker optionally having one or more carbon atoms replaced with N, O, S, SO or SO₂ and optionally being substituted with hydroxyl, mercapto, halogen, amino, aminoalkyl, nitro, oxo or thio. In a preferred embodiment X will have at least one carbon atom. Replacements and substitutions may form an amide moiety (-NRC(O)- or -C(O)NR-) within the hydrocarbon chain or at either or both ends. Other moieties include sulfonamide (-NRSO₂- or -SO₂NR), acyl, ether, thioether and amine. In a particularly preferred embodiment X is the group -CH₂-NR₆-C(O)- wherein the carbonyl -C(O)- portion thereof is adjacent (i.e. covalently bound) to Cy and R₆ is alkyl i.e. methyl and more preferably H.

30

Y is a carbocycle or heterocycle optionally substituted with hydroxyl, mercapto, halogen, oxo, thio, a hydrocarbon, a halo-substituted hydrocarbon, amino, amidine, guanidine, cyano, nitro, alkoxy or acyl. In particular embodiment, Y is aryl or heteroaryl optionally substituted with halogen or hydroxyl. In a particularly preferred embodiment, Y is phenyl, furan-2-yl, thiophene-

5 substituted moiety. When more than one, the substituents
may be the same or different group.

Cy is a non-aromatic carbocycle or heterocycle optionally substituted with hydroxyl (-OH), mercapto (-SH), thioalkyl, halogen (e.g. F, Cl, Br, I), oxo (=O), thio (=S), amino, aminoalkyl, amidine (-C(NH)-NH₂), guanidine (-NH₂-C(NH)-NH₂), nitro, alkyl or alkoxy. In a particular embodiment, Cy is a 3-5 member ring. In a preferred embodiment, Cy is a 5- or 6-member non-aromatic heterocycle optionally substituted with hydroxyl, mercapto, halogen (preferably F or Cl), oxo (=O), thio (=S), amino, amidine, guanidine, nitro, alkyl or alkoxy. In a more preferred embodiment, Cy is a 5-member non-aromatic heterocycle optionally substituted with hydroxyl, oxo, thio, Cl, C₁₋₄ alkyl (preferably methyl), or C₁₋₄ alkanoyl (preferably acetyl, propanoyl or butanoyl). More preferably the non-aromatic heterocycle comprises one or heteroatoms (N, O or S) and is optionally substituted with hydroxyl, oxo, mercapto, thio, methyl, acetyl, propanoyl or butyl. In particular embodiments the non-aromatic heterocycle comprises at least one nitrogen atom that is optionally substituted with methyl or acetyl. In a particularly preferred embodiment, the non-aromatic heterocycle is selected from the group consisting of piperidine, piperazine, morpholine, tetrahydrofuran, tetrahydrothiophene, oxazolidine, thiazolidine optionally substituted with hydroxy, oxo, mercapto, thio, alkyl or alkanoyl. In a most preferred embodiment Cy is a non-aromatic heterocycle selected from the group consisting of tetrahydrofuran-2-yl, thiazolidin-5-yl, thiazolidin-2-one-5-yl, and thiazolidin-2-thione-5-yl and cyclopropapyrrolidine.

5 The term "carbocycle" refers to a mono-, bi- or tri-cyclic carbon ring or ring system having 4-16 members (including bridged) which is saturated, unsaturated or partially unsaturated including aromatic (aryl) ring systems (unless specified as non-aromatic). Preferred
10 non-aromatic carbocyclic rings include cyclopropyl, cyclopropenyl, cyclobutyl, cyclobutenyl, cyclopentyl, cyclopentenyl, cyclohexyl and cyclohexenyl. Preferred aromatic carbocyclic rings include phenyl and naphthyl.

15 The term "heterocycle" refers to a mono-, bi- or tri-cyclic ring system having 5-16 members wherein at least one ring atom is a heteroatom (i.e. N, O and S as well as SO, or SO₂). The ring system is saturated, unsaturated or partially unsaturated and may be aromatic (unless specified as non-aromatic). Exemplary heterocycles include piperidine, piperazine, pyridine, pyrazine, pyrimidine, pyridazine, morpholine, pyran, pyrole, furan, thiophene (thienyl), imidazole, pyrazole, thiazole, isothiazole, dithiazole, oxazole, isoxazole, dioxazole,
20 thiadiazole, oxadiazole, tetrazole, triazole, thiatriazole, oxatriazole, thiadiazole, oxadiazole, purine and benzofused derivatives thereof.

25 The term "hydrocarbon chain" refers to saturated, unsaturated, linear or branched carbon chains i.e. alkyl, alkenyl and alkynyl. Preferred hydrocarbon chains incorporate 1-12 carbon atoms, more preferably 1-6 and most preferably 1-4 carbon atoms i.e. methyl, ethyl, propyl, butyl and allyl.

30
35 The phrase "optionally substituted with" is understood to mean, unless otherwise stated, that one or more of the specified substituents is covalently attached to the

5 The term "non-aromatic" refers to carbocycle or heterocycle rings that do not have the properties which define aromaticity. For aromaticity, a ring must be planar, have p-orbitals that are perpendicular to the plane of the ring at each ring atom and satisfy the
10 Huckel rule where the number of pi electrons in the ring is $(4n+2)$ wherein n is an integer (i.e. the number of pi electrons is 2, 6, 10 or 14). Non-aromatic rings provided herein do not satisfy one or all of these criteria for aromaticity.

15 The term "alkoxy" as used herein includes saturated, i.e. O-alkyl, and unsaturated, i.e. O-alkenyl and O-alkynyl, group. Exemplary alkoxy groups include methoxy, ethoxy, propoxy, butoxy, i-butoxy, s-butoxy, t-butoxy, pentyloxy and hexyloxy.
20

25 The term "amino" refers to a primary ($-NH_2$), secondary ($-NHR$), tertiary ($-N(R)_2$) or quaternary ($-N^+(R)_4$) amine wherein R is a hydrocarbon chain, hydroxy, a carbocycle, a heterocycle or a hydrocarbon chain substituted with a carbocycle or heterocycle.

30 The term "amino acid" refers to naturally and non-naturally occurring α -(alpha), β -(beta), D- and L-amino acid residues. Non-natural amino acids include those having side chains other than those occurring in nature.

35 By "carboxyl" is meant herein to be a free acid $-COOH$ as well as esters thereof such as alkyl, aryl and aralkyl esters. Preferred esters are methyl, ethyl, propyl, butyl, i-butyl, s-butyl and t-butyl esters.

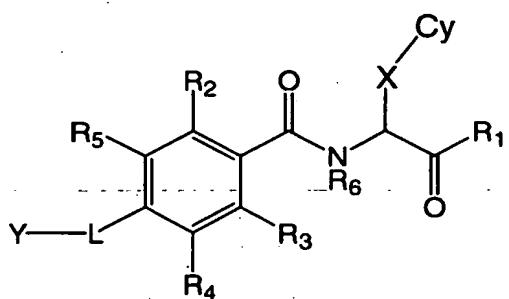
5 R₆ is H or a hydrocarbon chain optionally substituted with
a carbocycle or a heterocycle; and
salts, solvates and hydrates thereof;
with the proviso that when Y is phenyl, R₂, R₄ and R₅ are
H, R₃ is Cl and R₁ is OH then X is other than cyclohexyl.

10 In another aspect of the invention, there is provided
pharmaceutical compositions comprising a compound of the
invention and a pharmaceutically acceptable carrier.

15 In another aspect of the invention, there is provided a
method of treating a disease or condition mediated by
LFA-1 in a mammal comprising administering to said mammal
an effective amount of a compound of the invention.

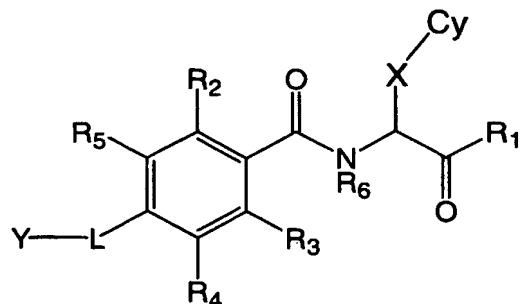
20 DETAILED DESCRIPTION OF THE INVENTION

The invention provides novel compounds of formula (I)



25 (I)

wherein Cy, X, Y, L and R₁₋₆ are as defined herein.
Compounds of the invention exhibit reduced plasma protein
binding affinity by virtue of a non-aromatic ring at
30 substituent Cy in comparison to those having an aromatic
ring at this portion of the molecule.



5

wherein

Cy is a non-aromatic carbocycle or heterocycle optionally substituted with hydroxyl, mercapto, thioalkyl, halogen, oxo, thio, amino, aminoalkyl, amidine, guanidine, nitro, alkyl, alkoxy or acyl;

X is a divalent hydrocarbon chain optionally substituted with hydroxyl, mercapto, halogen, amino, aminoalkyl, nitro, oxo or thio and optionally interrupted with N, O, S, SO or SO₂;

Y is a carbocycle or heterocycle optionally substituted with hydroxyl, mercapto, halogen, oxo, thio, a hydrocarbon, a halo-substituted hydrocarbon, amino, amidine, guanidine, cyano, nitro, alkoxy or acyl;

L is a bond or a divalent hydrocarbon optionally having one or more carbon atoms replaced with N, O, S, SO or SO₂ and optionally being substituted with hydroxyl, halogen oxo or thio; or three carbon atoms of the hydrocarbon are replaced with an amino acid residue;

R₁ is H, OH, amino, O-carbocycle or alkoxy optionally substituted with amino, a carbocycle or a heterocycle;

R₂₋₅ are independently H, hydroxyl, mercapto, halogen, cyano, amino, amidine, guanidine, nitro or alkoxy; or R₃ and R₄ together form a fused carbocycle or heterocycle optionally substituted with hydroxyl, halogen, oxo, thio, amino, amidine, guanidine or alkoxy;

5 mediated by LFA-1 including autoimmune diseases, graft
vs. host or host vs. graft rejection, and T-cell
inflammatory responses, so as to minimize side effects
and sustain specific tolerance to self- or xenoantigens.
There is also a need in the art to provide a non-peptide
10 antagonists to the LFA-1: ICAM-1 interaction.

Albumin is an abundant plasma protein which is responsible for the transport of fatty acids. However, albumin also binds and perturbs the pharmacokinetics of a wide range of drug compounds. Accordingly, a significant factor in the pharmacological profile of any drug is its binding characteristics with respect to serum plasma proteins such as albumin. A drug compound may have such great affinity for plasma proteins that it is not be available in serum to interact with its target tissue, cell or protein. For example, a compound for which 99% binds to plasma protein upon administration will have half the concentration available in plasma to interact with its target than a compound which binds only 98%.
25 Accordingly it would be desirable to provide LFA antagonist compounds which have low serum plasma protein binding affinity.

30 SUMMARY OF THE INVENTION

In an aspect of the present invention, there is provided novel compounds of formula (I)

5 use of anti-LFA-1 antibodies and ICAM-1, ICAM-2, and
ICAM-3 and their antibodies to treat LFA-1-mediated
disorders include WO 91/18011 published 11/28/91, WO
91/16928 published 11/14/91, WO 91/16927 published
11/14/91, Can. Pat. Appln. 2,008,368 published 6/13/91,
10 WO 90/03400, WO 90/15076 published 12/13/90, WO 90/10652
published 9/20/90, EP 387,668 published 9/19/90, WO
90/08187 published 7/26/90, WO 90/13281, WO 90/13316, WO
90/13281, WO 93/06864, WO 93/21953, WO 93/13210, WO
94/11400, EP 379,904 published 8/1/90, EP 346,078
15 published 12/13/89, U.S. Pat. No. 5,002,869, U.S. Pat.
No. 5,071,964, U.S. Pat. No. 5,209,928, U.S. Pat. No.
5,223,396, U.S. Pat. No. 5,235,049, U.S. Pat. No.
5,284,931, U.S. Pat. No. 5,288,854, U.S. Pat. No.
5,354,659, Australian Pat. Appln. 15518/88 published
20 11/10/88, EP 289,949 published 11/9/88, and EP 303,692
published 2/22/89, EP 365,837, EP 314,863, EP 319,815,
EP 468, 257, EP 362,526, EP 362, 531, EP 438,310.

25 Other disclosures on the use of LFA-1 and ICAM peptide
fragments and antagonists include; U.S. Pat. No.
5,149,780, U.S. Pat. No. 5,288,854, U.S. Pat. No.
5,340,800, U.S. Pat. No. 5,424,399, U.S. Pat. No.
5,470,953, WO 90/03400, WO 90/13316, WO 90/10652, WO
91/19511, WO 92/03473, WO 94/11400, WO 95/28170, JP
30 4193895, EP 314,863, EP 362,526 and EP 362,531.

35 The above methods successfully utilizing anti-LFA-1 or
anti-ICAM-1 antibodies, LFA-1 or ICAM-1 peptides,
fragments or peptide antagonists represent an
improvement over traditional immunosuppressive drug
therapy. These studies demonstrate that LFA-1 and ICAM-
1 are appropriate targets for antagonism. There is a
need in the art to better treat disorders that are

5 MAb, 25-3, was unable to control the course of acute
rejection in human kidney transplantation (LeMauff et
al., *Transplantation*, 52: 291 (1991)).

10 A review of the use of monoclonal antibodies in human
transplantation is provided by Dantil and Soulillou,
Current Opinion in Immunology, 3:740-747 (1991). An
earlier report showed that brief treatment with either
15 anti-LFA-1 or anti-ICAM-1 MAbs minimally prolonged the
survival of primarily vascularized heterotopic heart
allografts in mice (Isobe et al., *Science*, 255:1125
(1992)). However, combined treatment with both MAbs was
required to achieve long-term graft survival in this
model.

20 Independently, it was shown that treatment with anti-
LFA-1 MAb alone potently and effectively prolongs the
survival of heterotopic (ear-pinnae) nonprimarily
vascularized mouse heart grafts using a maximum dose of
4 mg/kg/day and treatment once a week after a daily dose
25 (Nakakura et al., *J. Heart Lung Transplant.*, 11:223
(1992)). Nonprimarily vascularized heart allografts are
more immunogenic and more resistant to prolongation of
survival by MAbs than primarily vascularized heart
allografts (Warren et al., *Transplant. Proc.*, 5:717
30 (1973); Trager et al., *Transplantation*, 47:587 (1989)).
The latter reference discusses treatment with L3T4
antibodies using a high initial dose and a lower
subsequent dose.

35 Another study on treating a sclerosis-type disease in
rodents using similar antibodies to those used by
Nakakura et al., *supra*, is reported by Yednock et al.,
Nature, 356:63-66 (1992). Additional disclosures on the

5

Experiments have also been carried out in primates. For example, based on experiments in monkeys it has been suggested that a MAb directed against ICAM-1 can prevent or even reverse kidney graft rejection (Cosimi et al., "Immunosuppression of Cynomolgus Recipients of Renal Allografts by R6.5, a Monoclonal Antibody to Intercellular Adhesion Molecule-1," in Springer et al. (eds.), *Leukocyte Adhesion Molecules* New York: Springer, (1988), p. 274; Cosimi et al., *J. Immunology*, 144:4604-4612 (1990)). Furthermore, the *in vivo* administration of anti-CD11a MAb to cynomolgus monkeys prolonged skin allograft survival (Berlin et al., *Transplantation*, 53: 840-849 (1992)).

20 The first successful use of a rat anti-murine CD11a antibody (25-3; IgG1) in children with inherited disease to prevent the rejection of bone-marrow-mismatched haploidentical grafts was reported by Fischer et al., *Lancet*, 2: 1058 (1986). Minimal side effects were observed. See also Fischer et al., *Blood*, 77: 249 (1991); van Dijken et al., *Transplantation*, 49:882 (1990); and Perez et al., *Bone Marrow Transplantation*, 4:379 (1989). Furthermore, the antibody 25-3 was effective in controlling steroid-resistant acute graft-versus-host disease in humans (Stoppa et al., *Transplant. Int.*, 4:3-7 (1991)).

35 However, these results were not reproducible in leukemic adult grafting with this MAb (Maraninchi et al., *Bone Marrow Transplant*, 4:147-150 (1989)), or with an anti-CD18 MAb, directed against the invariant chain of LFA-1, in another pilot study (Baume et al., *Transplantation*, 47: 472 (1989)). Furthermore, a rat anti-murine CD11a

5 function of LFA-1 (Davignon et al., *J. Immunol.*, 127:590
10 (1981)). LFA-1 is present only on leukocytes (Krenskey et al., *J. Immunol.*, 131:611 (1983)), and ICAM-1 is distributed on activated leukocytes, dermal fibroblasts, and endothelium (Dustin et al., *J. Immunol.* 137:245
15 (1986)).

Previous studies have investigated the effects of anti-CD11a MAbs on many T-cell-dependent immune functions *in vitro* and a limited number of immune responses *in vivo*.
15 In *vitro*, anti-CD11a MAbs inhibit T-cell activation (Kuypers et al., *Res. Immunol.*, 140:461 (1989)), T-cell-dependent B-cell proliferation and differentiation (Davignon et al., *supra*; Fischer et al., *J. Immunol.*, 136:3198 (1986)), target cell lysis by cytotoxic T-
20 lymphocytes (Krenskey et al., *supra*), formation of immune conjugates (Sanders et al., *J. Immunol.*, 137:2395 (1986); Mentzer et al., *J. Immunol.*, 135:9 (1985)), and the adhesion of T-cells to vascular endothelium (Lo et al., *J. Immunol.*, 143:3325 (1989)). Also, the antibody
25 5C6 directed against CD11b/CD18 was found to prevent intra-islet infiltration by both macrophages and T cells and to inhibit development of insulin-dependent diabetes mellitis in mice (Hutchings et al., *Nature*, 348: 639 (1990)).

30 The observation that LFA-1:ICAM-1 interaction is necessary to optimize T-cell function *in vitro*, and that anti-CD11a MAbs induce tolerance to protein antigens (Benjamin et al., *Eur. J. Immunol.*, 18:1079 (1988)) and prolongs tumor graft survival in mice (Heagy et al., *Transplantation*, 37: 520-523 (1984)) was the basis for testing the MAbs to these molecules for prevention of graft rejection in humans.
35

5 remain for bone marrow transplantation solve the problem
of immunoincompetence occurring when fully allogeneic
transplants are used.

As shown in Fig. 1, lymphocyte adherence to endothelium
10 is a key event in the process of inflammation. There
are at least three known pathways of lymphocyte
adherence to endothelium, depending on the activation
state of the T-cell and the endothelial cell. T-cell
immune recognition requires the contribution of the T-
15 cell receptor as well as adhesion receptors, which
promote attachment of - cells to antigen-presenting
cells and transduce regulatory signals for T-cell
activation. The lymphocyte function associated (LFA)
antigen-1 (LFA-1, CD11a/CD18, $\alpha_L\beta_2$: where α_L is CD11a
20 and β_2 is CD18) has been identified as the major
integrin receptor on lymphocytes involved in these cell
adherence interactions leading to several pathological
states. ICAM-1, the endothelial cell immunoglobulin-
25 like adhesion molecule, is a known ligand for LFA-1 and
is implicated directly in graft rejection, psoriasis,
and arthritis.

LFA-1 is required for a range of leukocyte functions,
including lymphokine production of helper T-cells in
30 response to antigen-presenting cells, killer T-cell-
mediated target cell lysis, and immunoglobulin
production through T-cell/B-cell interactions.
Activation of antigen receptors on T-cells and B-cells
allows LFA-1 to bind its ligand with higher affinity.

35 Monoclonal antibodies (MAbs) directed against LFA-1 led
to the initial identification and investigation of the

5

In some models such as vascular and kidney grafts, there exists a correlation between Class II matching and prolonged allograft survival, a correlation not present in skin grafts (Pescovitz et al., *J.Exp.Med.*, 160:1495-1508 (1984); Conti et al., *Transplant. Proc.*, 19: 652-654 (1987)). Therefore, donor-recipient HLA matching has been utilized. Additionally, blood transfusions prior to transplantation have been found to be effective (Opelz et al., *Transplant. Proc.*, 4: 253 (1973); Persijn et al., *Transplant. Proc.*, 23:396 (1979)). The combination of blood transfusion before transplantation, donor-recipient HLA matching, and immunosuppression therapy (cyclosporin A) after transplantation was found to improve significantly the rate of graft survival, and the effects were found to be additive (Opelz et al., *Transplant. Proc.*, 17:2179 (1985)).

The transplantation response may also be modified by antibodies directed at immune receptors for MHC antigens (Bluestone et al., *Immunol. Rev.* 90:5-27 (1986)). Further, graft survival can be prolonged in the presence of antigrift antibodies, which lead to a host reaction that in turn produces specific immunosuppression (Lancaster et al., *Nature*, 315: 336-337 (1985)). The immune response of the host to MHC antigens may be modified specifically by using bone marrow transplantation as a preparative procedure for organ grafting. Thus, anti-T-cell monoclonal antibodies are used to deplete mature T-cells from the donor marrow inoculum to allow bone marrow transplantation without incurring graft-versus-host disease (Mueller-Ruchholtz et al., *Transplant. Proc.*, 8:537-541 (1976)). In addition, elements of the host's lymphoid cells that

5 only the response to the donor alloantigen would be
lost). In addition, physicians specializing in
autoimmune disease strive for methods to suppress
autoimmune responsiveness so that only the response to
the self-antigen is lost. Such specific
10 immunosuppression generally has been achieved by
modifying either the antigenicity of the tissue to be
grafted or the specific cells capable of mediating
rejection. In certain instances, whether immunity or
tolerance will be induced depends on the manner in which
15 the antigen is presented to the immune system.

Pretreating the allograft tissues by growth in tissue culture before transplantation has been found in two murine model systems to lead to permanent acceptance across MHC barriers. Lafferty et al., *Transplantation*, 20 22:138-149 (1976); Bowen et al., *Lancet*, 2:585-586 (1979). It has been hypothesized that such treatment results in the depletion of passenger lymphoid cells and thus the absence of a stimulator cell population necessary for tissue immunogenicity. Lafferty et al., 25 *Annu. Rev. Immunol.*, 1:143 (1983). See also Lafferty et al., *Science*, 188:259-261 (1975) (thyroid held in organ culture), and Gores et al., *J. Immunol.*, 137:1482-1485 (1986) and Faustman et al., *Proc. Natl. Acad. Sci. U.S.A.*, 30 78: 5156-5159 (1981) (islet cells treated with murine anti-Ia antisera and complement before transplantation). Also, thyroids taken from donor animals pretreated with lymphocytotoxic drugs and gamma radiation and cultured for ten days *in vitro* were not rejected by any normal allogeneic recipient (Gose and Bach, *J. Exp. Med.*, 35 149:1254-1259 (1979)). All of these techniques involve depletion or removal of donor lymphocyte cells.

5 (azathioprine, bromocryptine, methylprednisolone,
prednisone, and most recently, cyclosporin A) have
significantly improved the clinical success of
transplantation. The nephrotoxicity of cyclosporin A
after renal transplantation has been reduced by co-
10 administration of steroids such as prednisolone, or
prednisolone in conjunction with azathioprine. In
addition, kidneys have been grafted successfully using
anti-lymphocyte globulin followed by cyclosporin A.
Another protocol being evaluated is total lymphoid
15 irradiation of the recipient prior to transplantation
followed by minimal immunosuppression after
transplantation.

20 Treatment of rejection has involved use of steroids, 2-
amino-6-aryl-5-substituted pyrimidines, heterologous
anti-lymphocyte globulin, and monoclonal antibodies to
various leukocyte populations, including OKT-3. See
generally *J. Pediatrics*, 111: 1004-1007 (1987), and
specifically U.S. Pat. No. 4,665,077.

25 The principal complication of immunosuppressive drugs is
infections. Additionally, systemic immunosuppression is
accompanied by undesirable toxic effects (e.g.,
nephrotoxicity when cyclosporin A is used after renal
30 transplantation) and reduction in the level of the
hemopoietic stem cells. Immunosuppressive drugs may
also lead to obesity, poor wound healing, steroid
hyperglycemia, steroid psychosis, leukopenia,
gastrointestinal bleeding, lymphoma, and hypertension.

35 In view of these complications, transplantation
immunologists have sought methods for suppressing immune
responsiveness in an antigen-specific manner (so that

5 itself. Nonsteroidal anti-inflammatory drugs are available, and many of them have effective analgesic, anti-pyretic and anti-inflammatory activity in RA patients. These include cyclosporin, indomethacin, phenylbutazone, phenylacetic acid derivatives such as 10 ibuprofen and fenoprofen, naphthalene acetic acids (naproxen), pyrrolealkanoic acid (tometin), indoleacetic acids (sulindac), halogenated anthranilic acid (meclofenamate sodium), piroxicam, and diflunisal. Other drugs for use in RA include anti-malarials such as 15 chloroquine, gold salts and penicillamine. These alternatives frequently produce severe side effects, including retinal lesions and kidney and bone marrow toxicity. Immunosuppressive agents such as methotrexate have been used only in the treatment of severe and 20 unremitting RA because of their toxicity. Corticosteroids also are responsible for undesirable side effects (e.g., cataracts, osteoporosis, and Cushing's disease syndrome) and are not well tolerated in many RA patients.

25 Another disorder mediated by T lymphocytes is host rejection of grafts after transplantation. Attempts to prolong the survival of transplanted allografts and xenografts, or to prevent host versus graft rejection, 30 both in experimental models and in medical practice, have centered mainly on the suppression of the immune apparatus of the host/recipient. This treatment has as its aim preventive immunosuppression and/or treatment of graft rejection. Examples of agents used for preventive 35 immunosuppression include cytotoxic drugs, anti-metabolites, corticosteroids, and anti-lymphocytic serum. Nonspecific immunosuppressive agents found particularly effective in preventive immunosuppression

5 adhesion (Loet al., *J. Immunol.* 143(10):3325-3329 (1989);
Smith et al., *J. Clin. Invest.* 83:2008-2017 (1989)) to
endothelial cells. Through the development of function
blocking monoclonal antibodies to ICAM-1 additional
ligands for LFA-1 were identified, ICAM-2 and ICAM-3
10 (Simmons, *Cancer Surveys* 24, Cell Adhesion and Cancer,
1995) that mediate the adhesion of lymphocytes to other
leukocytes as well as non-hematopoietic cells.
Interactions of LFA-1 with ICAM-2 are thought to mediate
natural killer cell activity (Helander et al., *Nature*
15 382:265-267 (1996)) and ICAM-3 binding is thought to play
a role in lymphocyte activation and the initiation of the
immune response (Simmons, *ibid*). The precise role of
these ligands in normal and aberrant immune responses
remains to be defined.

20

Disorders Mediated by T Lymphocytes

Function blocking monoclonal antibodies have shown that
LFA-1 is important in T-lymphocyte-mediated killing, T-
helper lymphocyte responses, natural killing, and
25 antibody-dependent killing (Springer et al., *Ann. Rev.
Immunol.* 5:223-252 (1987)). Adhesion to the target cell
as well as activation and signaling are steps that are
blocked by antibodies against LFA-1.

30

Many disorders and diseases are mediated through T
lymphocytes and treatment of these diseases have been
addressed through many routes. Rheumatoid arthritis
(RA) is one such disorder. Current therapy for RA
includes bed rest, application of heat, and drugs.
35 Salicylate is the currently preferred treatment drug,
particularly as other alternatives such as
immunosuppressive agents and adrenocorticosteroids can
cause greater morbidity than the underlying disease

5 regions have been identified, each with a typical length and having a consensus sequence of amino acid residues located between the cysteines of the disulfide bond (Williams, A. F. et al. *Ann Rev. Immunol.* 6:381-405 (1988); Hunkapillar, T. et al. *Adv. Immunol.* 44:1-63 (1989). ICAM-1 is expressed on a variety of 10 hematopoietic and non-hematopoietic cells and is upregulated at sites of inflammation by a variety of inflammatory mediators (Dustin et al., *J. Immunol.*, 137:256-254 (1986)). ICAM-1 is a 90,000-110,000 Mr 15 glycoprotein with a low messenger RNA levels and moderate surface expression on unstimulated endothelial cells. LPS, IL-1 and TNF strongly upregulate ICAM-1 mRNA and surface expression with peak expression at approximately 18-24 hours (Dustinet al., *J. Cell. Biol.* 107:321-331 (1988); Stauntonet al., *Cell* 52:925-933 (1988)). ICAM-1 20 has five extracellular Ig like domains (designated Domains 1, 2, 3, 4 and 5 or D1, D2, D3, D4 and D5) and an intracellular or cytoplasmic domain. The structures and sequence of the domains is described by Staunton et al. 25 (*Cell* 52:925-933 (1988)).

ICAM-1 was defined originally as a counter-receptor for LFA-1 (Springer et al., *Ann. Rev. Immunol.*, 5:223-252 (1987); Marlin*Cell* 51:813-819 (1987); Simmonset al., 30 *Nature* 331:624-627 (1988); Staunton*Nature* 339:61-64 (1989); Stauntonet al., *Cell* 52:925-933 (1988)). The LFA-1/ICAM-1 interaction is known to be at least partially responsible for lymphocyte adhesion (Dustinet al., *J. Cell. Biol.* 107:321-331 (1988); Mentzeret al., *J. Cell. Physiol.* 126:285-290 (1986)), monocyte adhesion 35 (Amaoutet al., *J. Cell Physiol.* 137:305 (1988); Mentzeret al., *J. Cell. Physiol.* 130:410-415 (1987); te Veldeet al., *Immunology* 61:261-267 (1987)), and neutrophil

5 β subunit. The β subunits are generally capable of
association with more than one α subunit and the
heterodimers sharing a common β subunit have been
classified as subfamilies within the integrin population
10 (Larson and Springer, "Structure and function of
leukocyte integrins," *Immunol. Rev.* 114:181-217 (1990)).

The integrin molecules of the CD11/CD18 family, and their
cellular ligands, have been found to mediate a variety of
cell-cell interactions, especially in inflammation.
15 These proteins have been demonstrated to be critical for
adhesive functions in the immune system (Kishimoto et al.,
Adv. Immunol. 46:149-182 (1989)). Monoclonal antibodies
to LFA-1 have been shown to block leukocyte adhesion to
endothelial cells (Dustin et al., *J. Cell. Biol.* 107:321-
20 331 (1988); Smith et al., *J. Clin. Invest.* 83:2008-2017
(1989)) and to inhibit T-cell activation (Kuypers et al.,
Res. Immunol., 140:461 (1989)), conjugate formation
required for antigen-specific CTL killing (Kishimoto et
25 al., *Adv. Immunol.* 46:149-182 (1989)), T cell
proliferation (Davignonet et al., *J. Immunol.* 127:590-595
(1981)) and NK cell killing (Krensky et al., *J. Immunol.*
30 131:611-616 (1983)).

ICAMs

30 ICAM-1 (CD54) is a cell surface adhesion receptor that is
a member of the immunoglobulin protein super-family
(Rothlein et al., *J. Immunol.* 137:1270-1274 (1986);
Staunton et al., *Cell* 52:925-933 (1988). Members of this
superfamily are characterized by the presence of one or
35 more Ig homology regions, each consisting of a disulfide-
bridged loop that has a number of anti-parallel β -pleated
strands arranged in two sheets. Three types of homology

5 *Suppl.*, 715:123 (1987); Weiss, S., *New England J. of
Med.*, 320:365 (1989)).

LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18)
The (CD11/CD18) family of adhesion receptor molecules
10 comprises four highly related cell surface glycoproteins;
LFA-1 (CD11a/CD18), Mac-1 (CD11b/CD18), p150.95
15 (CD11c/CD18) and (CD11d/CD18). LFA-1 is present on the
surface of all mature leukocytes except a subset of
macrophages and is considered the major lymphoid
integrin. The expression of Mac-1, p150.95 and
20 CD11d/CD18 is predominantly confined to cells of the
myeloid lineage (which include neutrophils, monocytes,
macrophage and mast cells). Functional studies have
suggested that LFA-1 interacts with several ligands,
25 including ICAM-1 (Rothlein et al., *J. Immunol.* 137:1270-
1274 (1986), ICAM-2, (Staunton et al., *Nature* 339:361-
364 (1989)), ICAM-3 (Fawcett et al., *Nature* 360:481-484
(1992); Vezeux et al., *Nature* 360:485-488, (1992); de
Fougerolles and Springer, *J. Exp. Med.* 175:185-190
25 (1990)) and Telencephalin (Tian et al., *J. Immunol.*
158:928-936 (1997)).

The CD11/CD18 family is related structurally and
genetically to the larger integrin family of receptors
30 that modulate cell adhesive interactions, which include;
embryogenesis, adhesion to extracellular substrates, and
cell differentiation (Hynes, R. O., *Cell* 48:549-554
(1987); Kishimoto et al., *Adv. Immunol.* 46:149-182 (1989);
Kishimoto et al., *Cell* 48:681-690 (1987); Ruoslahti et al.,
35 *Science* 238:491-497 (1987).

Integrins are a class of membrane-spanning heterodimers
comprising an α subunit in noncovalent association with a

5 leukocytes and other cell types. The binding of LFA-1 to
ICAMs mediate a range of lymphocyte functions including
lymphokine production of helper T-cells in response to
antigen presenting cells, T-lymphocyte mediated target
cells lysis, natural killing of tumor cells, and
10 immunoglobulin production through T-cell-B-cell
interactions. Thus, many facets of lymphocyte function
involve the interaction of the LFA-1 integrin and its
ICAM ligands. These LFA-1:ICAM mediated interactions
have been directly implicated in numerous inflammatory
15 disease states including; graft rejection, dermatitis,
psoriasis, asthma and rheumatoid arthritis.

20 While LFA-1 (CD11a/CD18) on lymphocytes plays a key role
in chronic inflammation and immune responses, other
members of the leukocyte integrin family (CD11b/CD18,
CD11c/CD18 and CD11d/CD18) also play important roles on
other leukocytes, such as granulocytes and monocytes,
particularly in early response to infective agents and in
acute inflammatory response.

25 The primary function of polymorphonuclear leukocytes,
derived from the neutrophil, eosinophil and basophil
lineage, is to sense inflammatory stimuli and to
emigrate across the endothelial barrier and carry out
30 scavenger function as a first line of host defense. The
integrin Mac-1(CD11b/CD18) is rapidly upregulated on
these cells upon activation and binding to its multiple
ligands which results in the release of oxygen derived
free radicals, protease's and phospholipases. In certain
35 chronic inflammatory states this recruitment is
improperly regulated resulting in significant cellular
and tissue injury. (Harlan, J. M., *Acta Med Scand* ~

5 large single nucleus and these cells may in turn become macrophages. Phagocytes are important in defending the host against a variety of infections and together with lymphocytes are also involved in inflammatory disorders.
10 The neutrophil is the most common leukocyte found in human peripheral blood followed closely by the lymphocyte. In a microliter of normal human peripheral blood, there are about 6,000 leukocytes, of which about 4,000 are neutrophils, 1500 are lymphocytes, 250 are monocytes, 150 are eosinophils and 25 are basophils.

15 During an inflammatory response peripheral blood leukocytes are recruited to the site of inflammation or injury by a series of specific cellular interactions (see Fig. 1). The initiation and maintenance of immune functions are regulated by intercellular adhesive interactions as well as signal transduction resulting from interactions between leukocytes and other cells.
20 Leukocyte adhesion to vascular endothelium and migration from the circulation to sites of inflammation is a critical step in the inflammatory response (Fig. 1). T-cell lymphocyte immune recognition requires the interaction of the T-cell receptor with antigen (in combination with the major histocompatibility complex) as well as adhesion receptors, which promote attachment of
25 T-cells to antigen-presenting cells and transduce signals for T-cell activation. The lymphocyte function associated antigen-1 (LFA-1) has been identified as the major integrin that mediates lymphocyte adhesion and activation leading to a normal immune response, as well as several pathological states (Springer, T.A., *Nature* 346:425-434 (1990)). Intercellular adhesion molecules (ICAM) -1, -2, and -3, members of the immunoglobulin superfamily, are ligands for LFA-1 found on endothelium,

LFA-1 ANTAGONIST COMPOUNDS

FIELD OF THE INVENTION

The invention relates to novel compounds which bind CD11/CD18 adhesion receptors, in particular Lymphocyte Function-associated Antigen-1 (LFA-1) as well as pharmaceutical compositions containing these compounds which are useful for treating disorders mediated thereby.

BACKGROUND OF THE INVENTION

Inflammation

Human peripheral blood is composed principally of red blood cells, platelets and white blood cells or leukocytes. The family of leukocytes are further classified as neutrophils, lymphocytes (mostly B- and T-cell subtypes), monocytes, eosinophils and basophils. Neutrophils, eosinophils and basophils are sometimes referred to as "granulocytes" or "polymorphonuclear (PMN) granulocytes" because of the appearance of granules in their cytoplasm and their multiple nuclei. Granulocytes and monocytes are often classified as "phagocytes" because of their ability to phagocytose or ingest micro-organisms and foreign mater referred to generally as "antigens". Monocytes are so called because of their

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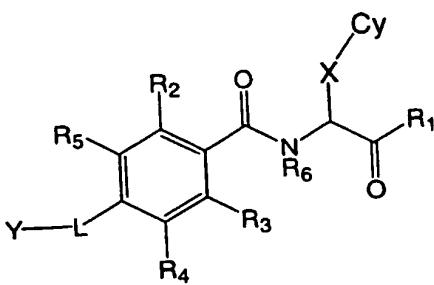
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A1

(54) Title: LFA-1 ANTAGONIST COMPOUNDS



(1)

(57) Abstract: The invention relates to novel compounds having formula (I), wherein Cy, X, Y, L and R₁-6 are as defined herein. The compounds bind CD11/CD18 adhesion receptors such as Lymphocyte Function-associated Antigen-1 (LFA-1) and are therefore useful for treating disorders mediated by LFA-1 such as inflammation

WO 02/059114 A1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 01/44203

Patent document cited in search report	Publication date		Patent family member(s)	Publication date
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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1,2,3,6,7,9-20 Completely:4,5,8

Present claims 1 to 3 relate to an extremely large number of possible compounds. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a small proportion of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the compounds wherein C_y is nearer defined (namely according to claims 4, 5 and 8 or description page 21, lines 5 to 27).

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.